UNIVERSITY OF ALBERTA

SYNTHETIC STUDIES ON ANGIOTENSIN-CONVERTING ENZYME INHIBITORS, PYRIDINES, AND PEPTIDES

by

Don M. Coltart ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Fall 2000

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-59572-2

Canadä

UNIVERSITY OF ALBERTA

LIBRARY RELEASE FORM

NAME OF AUTHOR:	Don M. Coltart
TITLE OF THESIS:	Synthetic Studies on Angiotensin-Converting Enzyme Inhibitors, Pyridines, and Peptides
DEGREE:	Doctor of Philosophy
YEAR THIS DEGREE GRANTED:	2000

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

1 Chiltern Place
Winnipeg, Manitoba
R3R 1T7

DATE: August 28, 2000

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Synthetic Studies on Angiotensin-Converting Enzyme Inhibitors, Pyridines, and Peptides submitted by Don M. Coltart in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

D. L. J. Clive

Dr. D. L. J. Clive

Dr. R. Tykwinski

Dr. G.Loppnow

Dr. N. Branda

Laura Frost

Dr. L. Frost

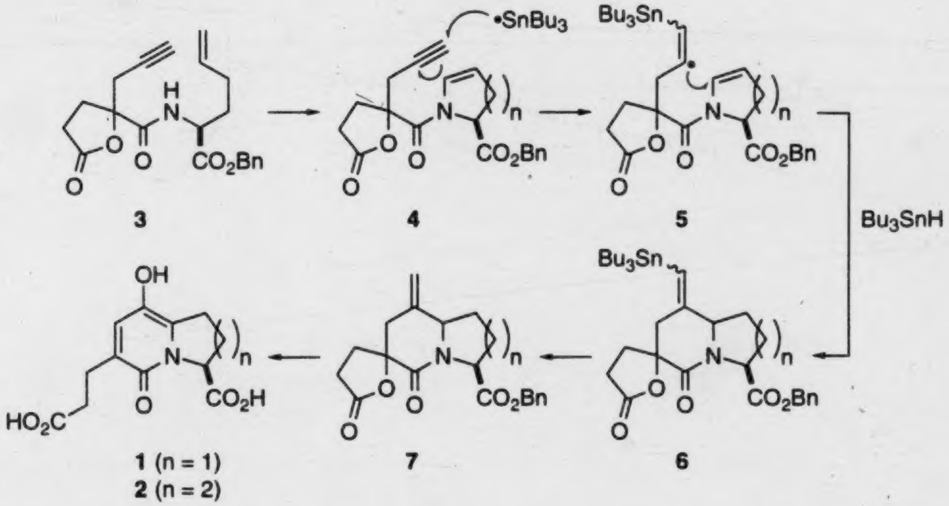
Aug. 24/2000

Dr. V. Snieckus

(External Examiner)

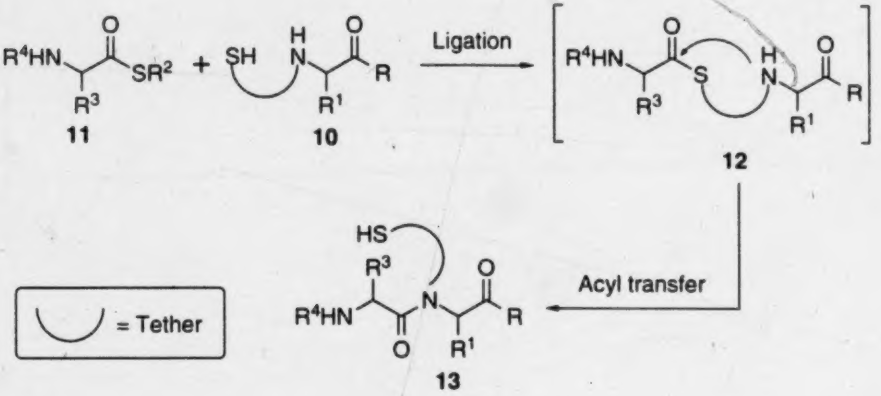
ABSTRACT

The first chapter of this thesis describes the total synthesis of the angiotensin-converting enzyme inhibitors A58365A (**1**) and A58365B (**2**), in both racemic and optically pure form. The synthesis of each compound was achieved from the advanced intermediate **3**. This species was elaborated to both the five and six-membered enamides **4**, which subsequently underwent radical cyclization to afford vinyl stannanes **6**. Protodestannylation of **6** gave the exocyclic olefins **7**, which were then elaborated to the target compounds **1** and **2**. The optical purity of the nonracemic final products was established by analytical HPLC using a chiral, nonracemic stationary phase.

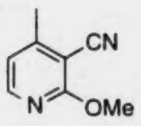


The final chapter of this thesis describes our preliminary investigations into peptide segment coupling by prior ligation and proximity-induced intramolecular acyl transfer. Several compounds of the general type **10** were prepared, each having a different thiol tether attached to the terminal amino group. These underwent ligation by thioester exchange with a thioester (**11**) to give an intermediate (**12**) in which the appropriate amino and acyl groups were in proximity. Intramolecular acyl transfer could then occur, thereby

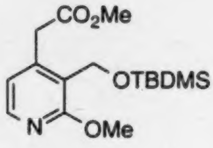
coupling the two components, by formation of a new peptide bond, to give **13**.



The appendix of this thesis describes the synthesis of 2-methoxy-4-methylpyridine-3-carbonitrile (**8**) and its conversion, by way of alkylation of the C-4 methyl group, into the pyridyl acetic ester **9**.



8



9

Dedicated to the memory of

Bonnie Sue Hohenstein

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. D. L. J. Clive for his support, encouragement, and advice during the course of my Ph.D. program, and for his assistance during the preparation of this thesis.

I would also like to thank my friends and colleagues for many helpful discussions, and for their support. In particular, I would like to acknowledge Dr. M. Sannigrahi and Dr. D. Mayhew for their generous assistance during the initial stages of my laboratory work, and Dr. Yuanxi Zhou for her advice on the ACE inhibitors project.

I gratefully acknowledge the financial assistance provided by the Killam Foundation, the Alberta Heritage foundation for Medical Research, and the Province of Alberta.

Lastly, I would like to offer a very special thank you to Jennifer Giordano, to my parents, and to my sister and her family for their constant support and encouragement.

TABLE OF CONTENTS

CHAPTER I

SYNTHESIS OF THE ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (–)-A58365A AND (–)-A58365B FROM A COMMON INTERMEDIATE

1 **INTRODUCTION** 2

1.1 Renin and Angiotensin 2

1.1.1 The Renin-Angiotensin System 2

1.1.2 Inhibitors of the Renin-Angiotensin System 4

(a) Angiotensin II Antagonists 4

(b) Angiotensin-Converting Enzyme Inhibitors 5

1.2 (–)-A58365A and (–)-A58365B 6

1.3 Other Syntheses of A58365A and A58365B 7

1.3.1 Total Synthesis of (–)-A58365A by Danishefsky and Fang 7

1.3.2 Total Synthesis of (±)-A58365B and Formal Synthesis of (±)-and (–)-A58365A by Moeller and Wong 10

(a) Formal Synthesis of (±)-A58365A 11

(b) Formal Synthesis of (–)-A58365A 13

(c) Total Synthesis of (±)-A58365B 15

1.3.3 Formal Synthesis of (–)-A58365A by Padwa and Straub 18

2 **RESULTS AND DISCUSSION** 20

2.1 Prior Attempted Synthetic Approaches to A58365A and A58365B 20

2.2 Successful Synthetic Approach to A58365A and A58365B 27

2.2.1 The Revised Strategy 27

2.2.2 Synthesis of (±)-A58365A and (±)-A58365B 30

(a) Preparation of the Advanced Intermediate for the Synthesis of (±)-A58365A and (±)-A58365B 30

(b) Elaboration of the Racemic Advanced Intermediate to (±)-A58365A 32

(c) Elaboration of the Racemic Advanced Intermediate to (±)-A58365B 35

2.2.3 Total Synthesis of (–)-A58365A and (–)-A58365B 37

(a) Preparation of the Advanced Intermediate for the Synthesis of (–)-A58365A and (–)-A58365B 37

(b) *Elaboration of the Optically Active Advanced Intermediate to (–)-A58365A* 38
(c) *Elaboration of the Optically Active Advanced Intermediate to (–)-A58365B* 40
(d) *Determination of the Enantiomeric Purity of Synthetic(–)-A58365A and (–)-A58365B* 42
3 CONCLUSION 46
4 FUTURE RESEARCH 46
5 EXPERIMENTAL 47
6 REFERENCES AND FOOTNOTES 128

CHAPTER 2

STUDIES ON PEPTIDE SEGMENT COUPLING BY PRIOR LIGATION AND PROXIMITY-INDUCED INTRAMOLECULAR ACYL TRANSFER

1 INTRODUCTION 133
1.1 *Background* 133
1.2 *Significance of Prior Ligation and Proximity-Induced Intramolecular Acyl Transfer* 135
1.3 *Prior Amine Capture Strategies* 139
  1.3.1 *Ligation by Hemiaminal Formation* 140
  1.3.2 *Ligation by Imine Formation* 144
  1.3.3 *Ligation by Michael Addition* 149
1.4 *Prior Thiol Capture Strategies* 152
  1.4.1 *Ligation by Mercaptide Formation* 155
  1.4.2 *Ligation by Disulfide Exchange* 156
    (a) *Template Design* 156
      (i) *Effective Molarity* 156
      (ii) *Transition State Model* 157
      (iii) *Evaluation of Templates* 159
    (b) *Effect of Amino Acid Side-Chains on the Rate of Intramolecular Acyl Transfer* 165
    (c) *Extent of Epimerization* 166
    (d) *Disulfide Formation* 167
    (e) *Synthesis of the Dibenzofuran-Linked N-Terminal Peptide Segments* 170

(f) Cysteine Thiol Protection and a Modified Prior Thiol Capture Strategy 173
(g) Application to Peptide Synthesis 175
1.5 Native Chemical Ligation Strategies 178
1.5.1 Ligation by Thioester Exchange Involving Cysteine 179
(a) Mechanistic and Practical Aspects 180
(b) Extent of Epimerization 184
(c) Synthesis of Peptide Segments 184
(d) Application to Peptide Synthesis 185
(e) Application to the Solid Phase (Solid Phase Chemical Ligation) 188
(i) Solid Phase Chemical Ligation in the N to C direction 189
(ii) Solid Phase Chemical Ligation in the C to N direction 191
1.5.2 Ligation by Thioester Exchange Involving Derivatized Glycine or Alanine 193
1.6 Orthogonal Ligation Coupling Strategies 197
1.6.1 Ligation by Thiazolidine Formation 197
(a) Mechanistic and Model Studies 198
(b) Synthesis of Peptide Segments 201
(c) Application to Peptide Synthesis 203
1.6.2 Ligation by Thioester Exchange 205
1.6.3 Ligation by Thioester Formation 207
1.6.4 Ligation by Disulfide Exchange 210
1.6.5 Ligation by Amide Formation 211
2 RESULTS AND DISCUSSION 214
2.1 Background 214
2.2 Segment Coupling Approaches Involving Ligation by Thioester Exchange 215
2.2.1 Research Objectives 215
(a) Development of a Generalized Approach to Peptide Segment Coupling 215
(b) Development of a Generalized Iterative Approach to Peptide Segment Coupling 218
(c) Adaptation to the Solid Phase 221

(i) *Solid Phase Segment Coupling in the N to C direction* 222
(ii) *Solid Phase Segment Coupling in the C to N direction* 225
2.2.2 *Considerations Regarding the Tether* 228
(a) *Tether Cleavage* 228
(b) *Thiol Nucleophilicity* 231
(c) *Transition State Size and Shape* 232
2.2.3 *Studies Involving Benzylic Tether* ***80.1*** 233
(a) *Synthesis and Examination of a Model System for Acyl Transfer* 233
(b) *Synthesis of the Specially Derivatized Amino Acid* 239
(c) *Deprotection, Ligation, and Intramolecular Acyl Transfer* 250
(d) *Studies Towards an Iterative Approach* 257
2.2.4 *Studies Involving Benzylic Tether* ***80.2*** 258
(a) *Synthesis of a Model System for Ligation and Acyl Transfer* 258
(b) *Ligation and Acyl Transfer* 263
2.2.5 *Studies Involving Dimethyl Tether* ***80.3*** 274
(a) *Synthesis of a Model System for Ligation and Acyl Transfer* 274
(b) *Ligation and Acyl Transfer* 275
2.3 *Segment Coupling Approaches Involving Nucleophilic Acylation Catalyst Derivatives* 276
2.3.1 *Proposed Research* 276
2.3.2 *Studies Involving DMAP-based Tether* ***131.1*** 278
(a) *Synthesis of a Model System for Ligation and Acyl Transfer* 278
(b) *Ligation and Acyl Transfer* 279
3 **CONCLUSION** 281
4 **FUTURE RESEARCH** 282
4.1 *Segment Coupling Approaches Involving Ligation by Thioester Exchange* 282
4.2 *Segment Coupling Approaches Involving Nucleophilic Acylation Catalyst Derivatives* 285

5 EXPERIMENTAL 285
6 REFERENCES AND FOOTNOTES 310

APPENDIX 1

STUDIES ON THE PREPARATION OF 3,4-DISUBSTITUTED 2-METHOXYPYRIDINES

A.1 INTRODUCTION 320
A.2 RESULTS AND DISCUSSION 321
A.3 CONCLUSION 326
A.4 EXPERIMENTAL 326
A.5 REFERENCES AND FOOTNOTES 337

LIST OF TABLES

CHAPTER 3

Table 1 Effect of Solvent on the Product Distribution for Reaction of **4.1** with Benzylamine

Table 2 Product Distribution when **5.1** Reacts with Different Amines

Table 3 Half-Times for Intramolecular Acyl Transfer Reactions of **7.4**

Table 4 Half-Times for Intramolecular Acyl Transfer Reactions of **9.6a,b**

Table 5 Half-Times for Intramolecular Acyl Transfer Reactions of **10.2a-c**

Table 6 Rates of Acyl Transfer in **11.2**

Table 7 Half-Times for Intramolecular Acyl Transfer Reactions of **27.1**

Table 8 Effect of *N*-Terminal Residue (X) of *C*-Terminal Segment on Coupling of LYRAX-SR and CRANK by *Native Chemical Ligation*

Table 9 Model peptides Used to Study the Approach of Scheme 53

Table 10 Results of Model Study for the Approach of Scheme 53

Table 11 Heterocyclic Ligation Products Resulting from Reaction Between Gylcoaldehyde-Derivatized Peptides and Various *N*-Terminal Residues

Table 12 Half-Times for Acyl Transfer of **56.5a,d,e** at Various pHs

Table 13 Kinetic Data of synthetic HIV-1 Protease Analogs Compared to Wild Type

Table 14 Product distribution for Reaction Between **62.2** and **62.3**

Table 15 Product Distribution for Reaction of **69.1a-c** with **69.2a,b**

Table 16 Conditions Used and Half-Times Observed for Model Coupling Reactions for the Formation of **123.6** Conducted at Approximately 100 mM Concentration

Table 17 Conditions Used and Half-Times Observed for Model Coupling Reactions for the Formation of **123.6** Conducted at Approximately 10 mM Concentration

LIST OF ABBREVIATIONS

AIBN	2,2'-azobisisobutyronitrile
9-BBN	9-borabicyclo[3.3.1]nonane
Boc	*tert*-butoxycarbonyl
Bn	benzyl
t-Bu	*tert*-butyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride
Fm	9-fluroenylmethyl
Fmoc	9-fluorenylmethylcarbonyl
LDA	lithium diisopropylamide
MCPBA	*m*-chloroperoxybenzoic acid
PCC	pyridinium chlorochromate
Ph	phenyl
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TMS	trimethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid

TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Ts	*p*-toluenesulfonyl

CHAPTER 1

SYNTHESIS OF THE ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (–)-A58365A AND (–)-A58365B FROM A COMMON INTERMEDIATE

1 INTRODUCTION

1.1 Renin and Angiotensin[1]

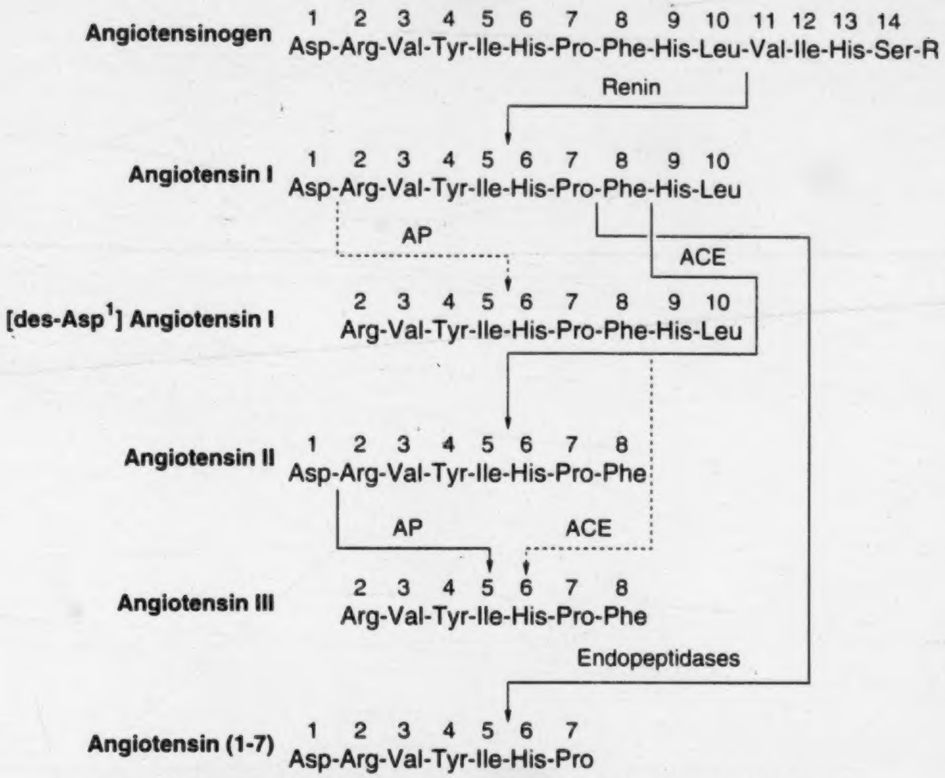
1.1.1 The Renin-Angiotensin System

The crude saline extracts of kidney contain a pressor principle known as renin, which is an enzyme that produces persistent hypertension (elevated blood pressure) in humans and animals by constricting the renal arteries. This occurs when renin acts on a plasma protein substrate, known as angiotensinogen, catalyzing the formation of the actual pressor material, which is a peptide called angiotensin.

In the 1950s, Skeggs and Peart determined the amino acid composition and sequence of angiotensin, and two forms were recognized. The first is a decapeptide (angiotensin I) and the second is an octapeptide (angiotensin II). The latter is formed from angiotensin I by enzymatic cleavage by another enzyme, termed angiotensin-converting enzyme (ACE). The octapeptide, angiotensin II, was shown to be the more active species, and its synthesis[2] in 1957 made the material available for intensive study.

The synthesis and degradation of the angiotensins is a complex process, and it is outlined in Scheme 1. Briefly, the process is initiated when the enzyme renin acts on angiotensinogen (the renin substrate) to release the decapeptide angiotensin I. This decapeptide has limited pharmacological activity, but it is cleaved by angiotensin-converting enzyme to yield the highly active octapeptide angiotensin II. This, in turn, undergoes hydrolysis by an aminopeptidase to yield the heptapeptide angiotensin III, which is also pharmacologically active. Further cleavage yields peptides with little activity. In an alternative and minor pathway, angiotensin-converting enzyme and aminopeptidase act in the opposite order such that the decapeptide, angiotensin I, is hydrolyzed first to [des-asp^{I}] angiotensin I which, like the parent compound, has limited pharmacological activity. The [des-asp^{I}] angiotensin I is then cleaved by angiotensin-converting enzyme to form the active angiotensin III.[2,3]

Scheme 1



AP = Aminopeptidase; ACE = Angiotensin-converting enzyme; Solid arrows indicate major pathways; Dashed arrows indicate minor pathways

There are many fatal diseases which are related to excessive amounts of angiotensin II in blood. The compound causes vasoconstriction and often has an indirect effect on the heart, such that cardiac output is lowered. Angiotensin II is the most potent pressor agent known; on a molar basis, it is about 40 times more powerful than norepinephrine. It also causes kidney disease due to its indirect effects on renal tubular function, mediated by aldosterone. Additionally, angiotensin influences urine formation through hemodynamic and intrarenal actions that interact in a complex way.

The pharmacological properties that have been described are those of angiotensin II. Angiotensin I has less than 1% of the intrinsic activity of angiotensin II. However, angiotensin III retains most of the activity of angiotensin II, although in most instances it is somewhat weaker.

1.1.2 Inhibitors of the Renin-Angiotensin System

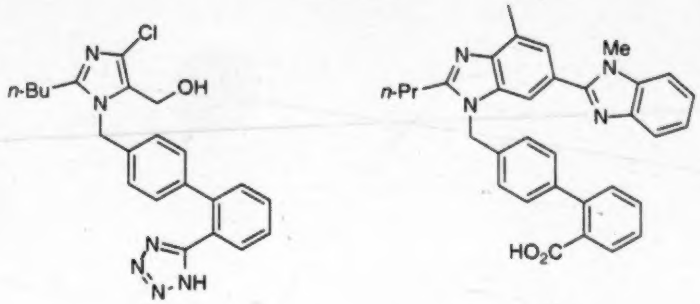
The renin-angiotensin system plays an important role in the control of renal function and blood pressure, and in the pathogenesis of some forms of hypertension. Consequently, much work has been focused on developing agents that block the renin-angiotensin system, so as to maintain blood pressure within the proper range.

In the 1970s, two distinct classes of effective inhibitors of the renin-angiotensin system were identified: angiotensin II antagonists, which block receptors for the peptide, and angiotensin-converting enzyme inhibitors, which slow the rate of formation of angiotensin II from its inactive precursor.

(a) Angiotensin II Antagonists

The useful antagonists of angiotensin II are slightly modified congeners of the natural structure, in which agonist activity is profoundly attenuated by replacement of phenylalanine in position 8 with some other amino acid. The substances [Sar^1, Val^5, Ala^8] angiotensin (1-8) octapeptides, known as the Saralasin series, were introduced by Pals in 1971. Although angiotensin II analogs are highly specific antagonists, they also retain some agonist activity, which complicates interpretation of some of their effects.[4] However, direct blockade of the angiotensin II receptor (AT_1 receptor) has recently become feasible by the development of a series of orally active nonpeptide antagonists for the AT_1 receptor. Losartan (**2.1**, Scheme 2) and Telmisartan (**2.2**) have been found to be very powerful AT_1 antagonists.[5,6,7] These nonpeptide antagonists are highly selective for the AT_1 receptor and, in contrast to the peptide-based antagonists of angiotensin analogs, they do not show any partial agonism.

Scheme 2



2.1
Losartan

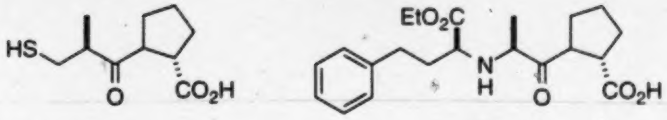
2.2
Telmisartan

(b) Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme inhibitors are also commonly used for inhibiting the renin-angiotensin system (RAS).[1] The essential effect of these agents on the RAS is to inhibit conversion of the relatively inactive angiotensin I into the active angiotensin II (or the conversion of [des-Asp^{I}] angiotensin I to angiotensin III). In this way they attenuate or abolish responses to angiotensin I. Angiotensin-converting enzyme inhibitors are highly specific drugs. They do not interact directly with other components of the renin-angiotensin system, including the receptor for the peptide. Also, research has revealed that angiotensin-converting enzyme inhibitors cause a greater decrease in blood pressure than does angiotensin II antagonism.

Captopril (**3.1**, Scheme 3),[8] the first orally active ACE inhibitor, has been marketed for the treatment of severe or drug-resistant hypertension since the 1980s. Unfortunately, it can cause some potentially hazardous side effects, possibly related to its sulfhydryl moiety.[9] Enalapril (**3.2**), a nonsulfhydryl-containing ACE inhibitor, is also used for treatment of diseases caused by angiotensin. Enalapril is a prodrug that is not itself highly active; it must be hydrolyzed to its active parent dicarboxylic acid, Enalaprilate. Enalapril has been found to be more potent than Captopril and to inhibit ACE for longer periods.[8,9,10]

Scheme 3



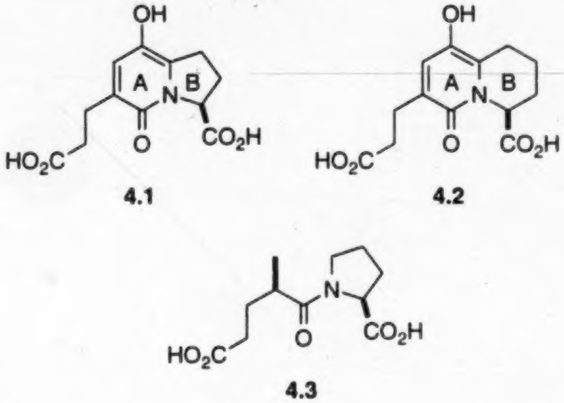
3.1
Captopril

3.2
Enalapril

1.2 (–)-A58365A and (–)-A58365B

Culture A58365, NRRL 15098, identified as a new strain of *Streptomyces chromofuscus*, was isolated from a Brazilian soil sample. It was found to produce the two novel angiotensin-converting enzyme inhibitors (–)-A58365A and (–)-A58365B. These species possess homologous nitrogen-containing bicyclic structures of molecular formulae $C_{12}H_{13}NO_6$ for (–)-A58365A and $C_{13}H_{15}NO_6$ for (–)-A58365B.[11,12]

Scheme 4



The structure elucidation of the two new ACE inhibitors has been described in detail.[13] (–)-A58365A was determined to be 3-carboxy-1,2,3,5-tetrahydro-8-hydroxy-5-oxo-6-indolizine-propanoic acid (**4.1**); (–)-A58365B was the homologous 4-carboxy-1,3,4,6-tetrahydro-9-hydroxy-6-oxo-2*H*-quinolizine-7-propanoic acid (**4.2**). (–)-A58365A is, in fact, a naturally occurring, conformationally restricted analog of α-methylglutaryl-L-proline (**4.3**), a compound which played a role in the structure activity relationship studies leading to the development of Captopril.

Nakatsukasa and coworkers have investigated the biosynthetic pathway for the formation of A58365A and A58365B by *Streptomyces chromofuscus* NRRL 15098.[14] Fermentation studies afforded an increase in the amount of the ACE inhibitor from less than 1 μg/mL to 20 μg/mL. Proline was the obligatory supplement for ACE inhibitor biosynthesis. Without proline, less than 1 μg/mL of both A58365A and A58365B was synthesized. D-proline or L-hydroxyproline could be substituted in place of L-proline, but were not superior to L-proline. Greater amounts of A58365A were synthesized when L-tyrosine was added to the medium, but only when added in combination with proline. The addition of lysine to the proline-supplemented medium resulted in the fermentation process producing greater amounts of A58365B but, again, only when added in combination with proline. This observation suggested that A58365B synthesis is closely linked to that of A58365A. The studies also showed that A58365A is synthesized prior to the formation of A58365B.

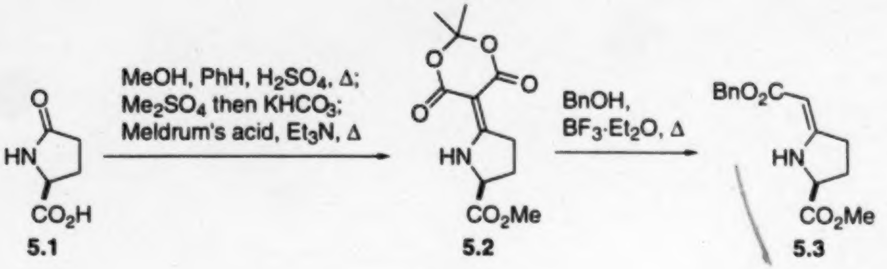
1.3 Other Syntheses of A58365A and A58365B

When we began our synthetic studies, a route to (–)-A58365A had already been published by Danishefsky and Fang[15] and, during the course of our work, the total synthesis of (±)-A58365B and formal syntheses of (±)- and (–)-A58365A were reported by Moeller and Wong.[16] Subsequent to our syntheses, a formal synthesis of (–)-A58365A was described by Padwa and Straub.[17]

1.3.1 Total Synthesis (–)-A58365A by Danishefsky and Fang[15]

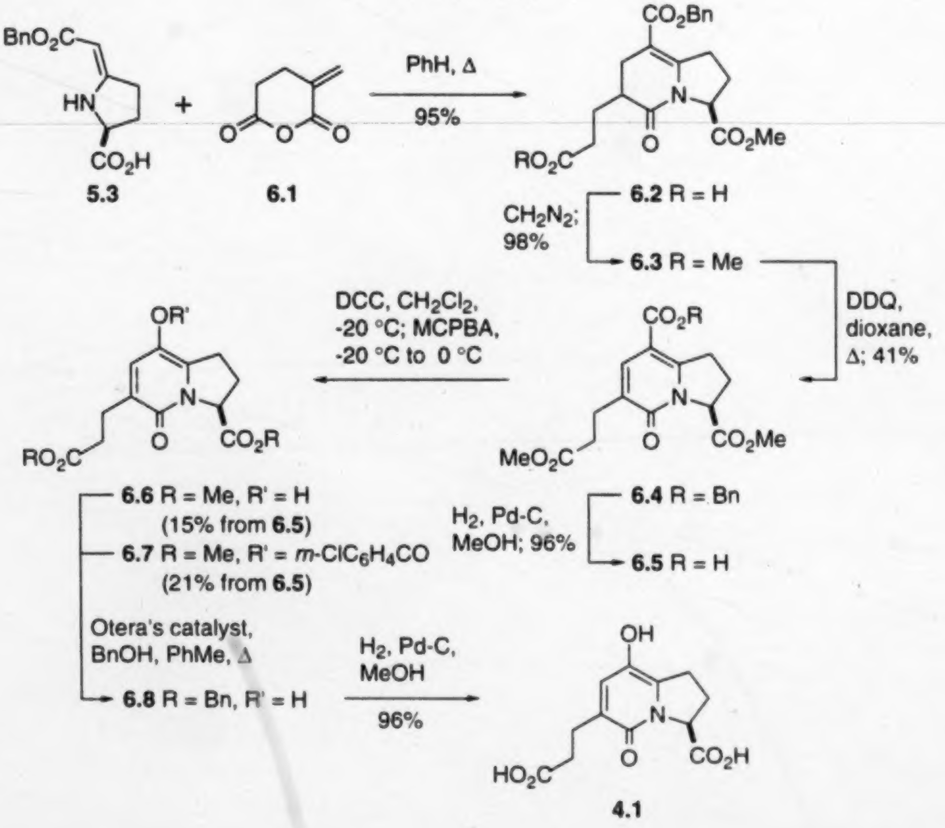
The first synthesis of optically pure (–)-A58365A was reported by Danishefsky and Fang in 1989. Their synthesis was accomplished starting from optically pure L-pyroglutamic acid (**5.1**) which was converted via a literature procedure into vinylogous urethane **5.3** (Scheme 5), a key intermediate in the synthesis. The annulation of **5.3** with

Scheme 5



α-methyleneglutaric anhydride (**6.1**, Scheme 6) generated hexahydroindolizidine **6.2**, in 95% yield. Esterification (**6.2** → **6.3**), followed by DDQ oxidization (**6.3** → **6.4**), then gave pyridone **6.4**, which was subsequently hydrogenolyzed to monocarboxylic acid **6.5**. Carboxy-inversion of **6.5** was achieved by successive treatment with DCC and MCPBA,

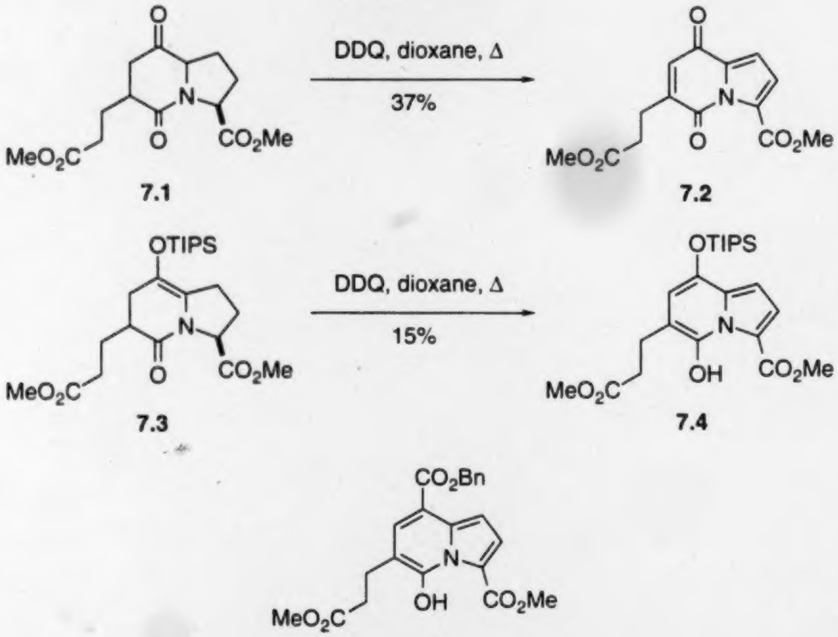
Scheme 6



giving A58365A-dimethyl ester (**6.6**) and A58365A-dimethyl ester *meta*-chlorobenzoate (**6.7**). In order to facilitate purification, **6.6** and **6.7** were converted into dibenzyl ester **6.8** by treatment with Otera's catalyst and BnOH, and **6.8** was subjected to hydrogenolysis to give the natural product, (–)-A58365A (**4.1**). On the basis of optical rotation data, the synthetic material was obtained in 100% ee.

As pointed out by Danishefsky in the original publication, the first generation synthesis of (–)-A58365A presented above involves some low yielding steps. One of these is the oxidation of **6.3** to **6.4**, using DDQ. No comment is made in the original publication but, by analogy to subsequently reported[16] related transformations (**7.1** → **7.2** and **7.3** → **7.4**, Scheme 7), it seems possible that the low yield obtained is a result of over oxidation of the desired product (**6.4**, Scheme 6) to a species of the type represented by structure **7.5**, which is a Hückel 4n + 2 π electron aromatic compound. Another potential weakness of the strategy is revealed by considering its application to the synthesis of the

Scheme 7

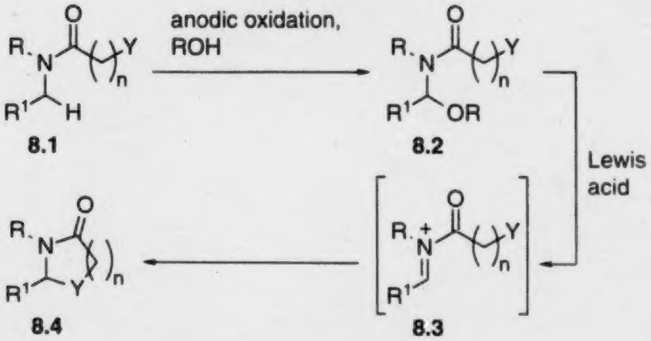


related [4.4.0] bicyclic analog A58365B. For the synthesis of Danishefsky and Fang to be applicable to A58365B, an optically pure six-membered analog of pyroglutamic acid (**5.1**) would have to be used. Unfortunately, no such six-membered analog is known that is *directly* available from the chiral pool, and so the route to A58365B would have to incorporate the additional steps necessary to obtain such an intermediate.

1.3.2 Total Synthesis of (±)-A58365B and Formal Synthesis of (±)- and (–)-A58365A by Moeller and Wong[16]

Moeller and Wong have achieved the total synthesis of (±)-A58365B and formal synthesis of (±)- and (–)-A58365A using, as a key transformation in each case, an anodic amide oxidation-iminium ion cyclization. The oxidation-cyclization strategy is outlined in a general way in Scheme 8. Here, an amide is initially oxidized, using an electrochemical process, at the position α to the nitrogen atom (**8.1** → **8.2**). An appropriate Lewis acid is then employed in order to generate an iminium ion (**8.2** → **8.3**) *in situ*, which is then captured by a pendant nucleophile (**8.3** → **8.4**). The net result of the process is annulation through the formation of a lactam, and thus it is a useful procedure for the construction of the B ring of A58365A and A58365B from appropriate intermediates.

Scheme 8

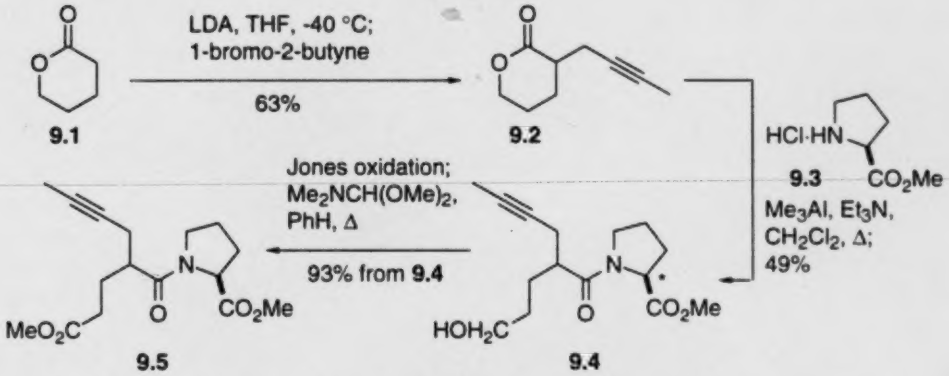


Y = nucleophile

(a) Formal Synthesis of (±)-A58365A

In the synthesis of (±)-A58365A, the key intermediate (**9.5**) for electrolysis was synthesized in four steps as outlined in Scheme 9. δ-Valerolactone (**9.1**) was alkylated with 1-bromo-2-butyne to give lactone **9.2**, which was then condensed with proline methyl ester hydrochloride (**9.3**), resulting in the formation of diastereomeric acetylenic amides **9.4**. The diastereomers were not separated but were used directly in the subsequent transformations. Since the stereogenic center α to the amide carbonyl of **9.4** would eventually become sp^2 hybridized, the fact that a mixture of diastereomers was used was not important to the outcome of the synthesis. Oxidation of the acetylenic amides **9.4**, followed by esterification, generated the required electrolysis substrate **9.5**.

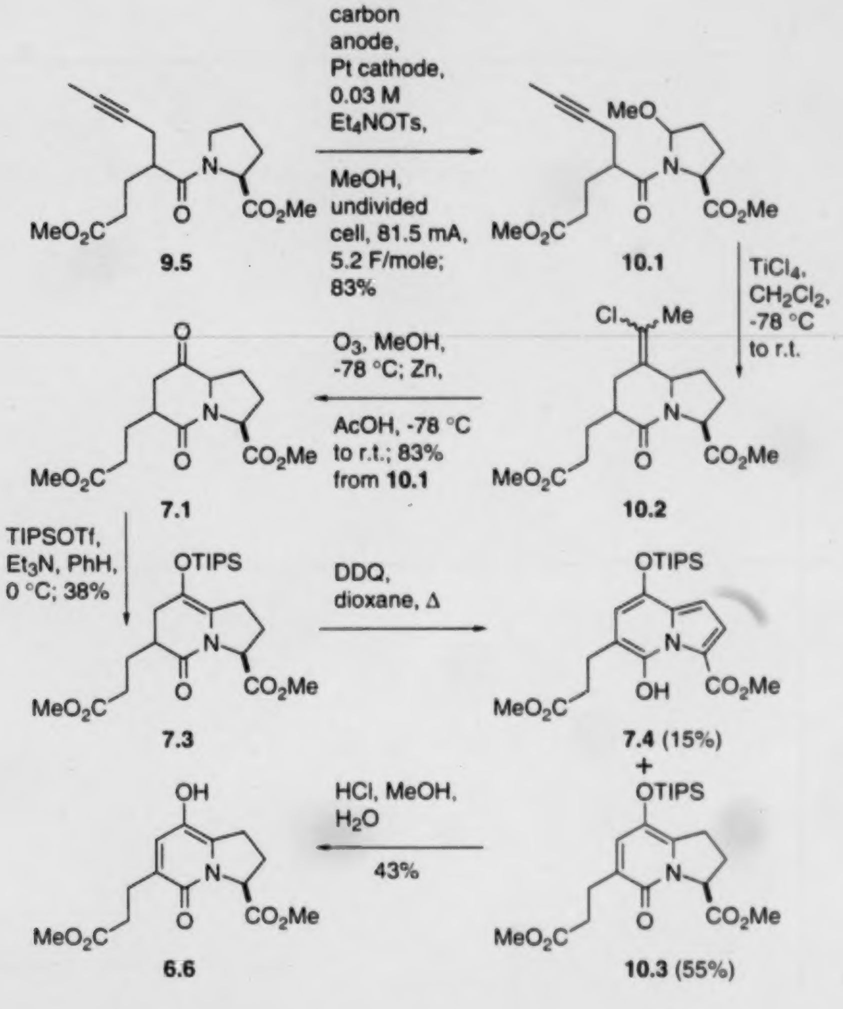
Scheme 9



*This center was later found to have undergone epimerization.

The diastereomeric mixture of compounds **9.5** was allowed to react under the optimized electrolytic oxidation conditions shown in Scheme 10, affording cyclization intermediates **10.1** in 83% yield. As before, the fact that a mixture of stereoisomers was obtained was of little consequence since the stereogenic centers involved would eventually be converted into sp^2 hybridization. Compounds **10.1** were cyclized in the presence of $TiCl_4$ and the resulting crude vinyl chlorides (**10.2**) were immediately ozonized to intermediates **7.1**. Attempted oxidation of these species with DDQ proved troublesome as only over-oxidized material was obtained (see **7.1** → **7.2**, Scheme 7). The problem of over-oxidation could be avoided to some extent by first converting **7.1** into triisopropylsilyl enol ethers **7.3** (**7.1** → **7.3**, Scheme 10). When these were treated with DDQ, the desired product (**10.3**) was obtained, albeit in modest (55%) yield, along with the corresponding over-oxidized compound **7.4**. The triisopropylsilyl group was then cleaved (**10.3** → **6.6**, Scheme 10), converging the synthetic route with the Danishefsky synthesis described above (*cf.* **6.6**, Scheme 6). The optical rotation of **6.6**, however,

Scheme 10


carbon anode, Pt cathode, 0.03 M Et_4NOTs,
MeOH, undivided cell, 81.5 mA, 5.2 F/mole; 83%
MeO
N
CO_2Me
MeO_2C
O
9.5
10.1
$TiCl_4$, CH_2Cl_2, -78 °C to r.t.
Cl
Me
10.2
O_3, MeOH, -78 °C; Zn,
AcOH, -78 °C to r.t.; 83% from 10.1
7.1
TIPSOTf, Et_3N, PhH, 0 °C; 38%
OTIPS
7.3
DDQ, dioxane, Δ
OH
7.4 (15%)
+
10.3 (55%)
HCl, MeOH, H_2O
43%
6.6

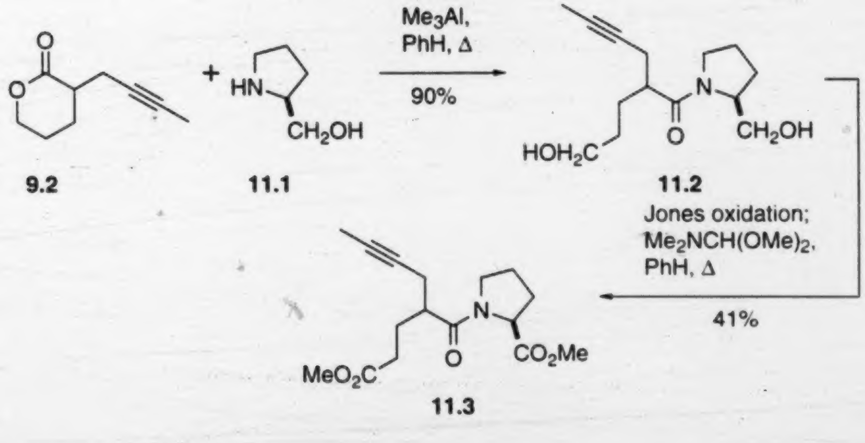
indicated that the synthetic material was racemic, implying that racemization of the stereogenic center derived from proline methyl ester hydrochloride (**9.3**) had occurred at some stage. Hence, the above work amounts to a formal synthesis of (±)-A58365A.

(b) Formal Synthesis of (–)-A58365A

Prior to embarking on a formal synthesis of optically active A58365A, a series of control experiments were carried out to deduce the origin of epimerization noted in the above route to (±)-A58365A. It was found that complete epimerization was occurring during the trimethylaluminium-catalyzed amide bond-forming reaction (**9.2** + **9.3** → **9.4**, Scheme 9). The authors were able to establish that the problem of epimerization at this center was easily avoided by conducting the reaction with (*S*)-(+)-2-pyrrolidinemethanol (**11.1**, Scheme 11) instead of proline methyl ester hydrochloride (**9.3**).

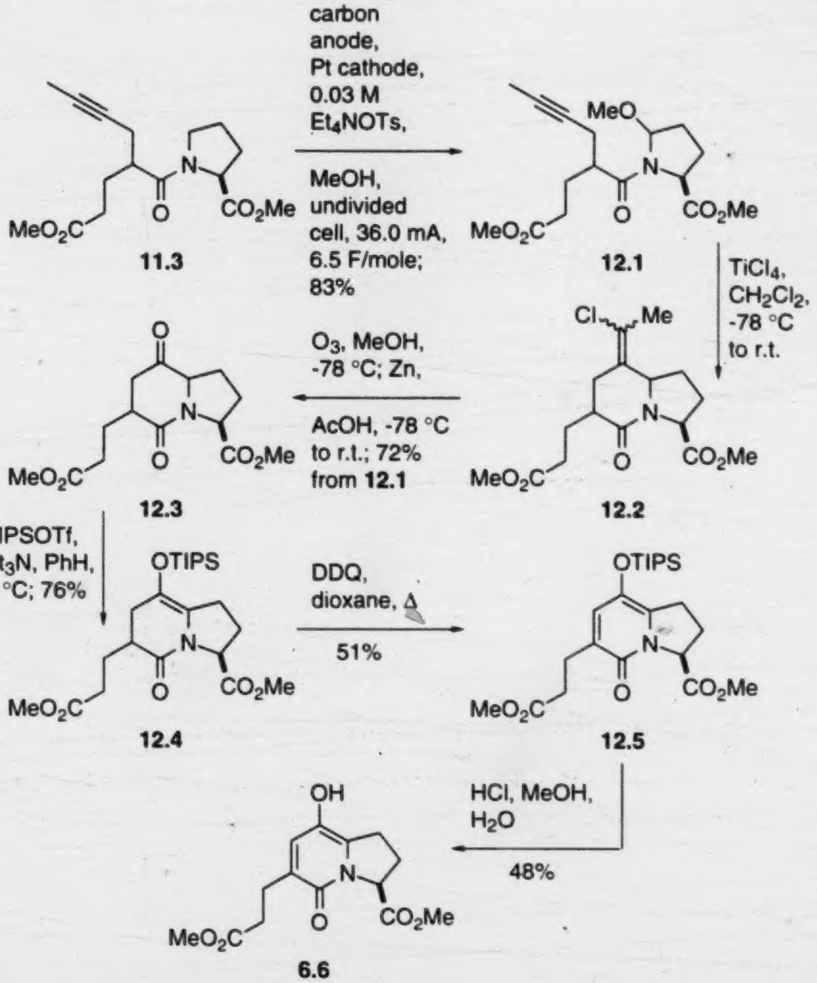
The electrolysis substrate required for the present synthesis was prepared as outlined in Scheme 11. Trimethylaluminium-mediated amide bond formation, this time between lactone **9.2** and (*S*)-(+)-2-pyrrolidinemethanol (**11.1**), proceeded smoothly to give compound **11.2**, as a mixture of diastereomers, in optically active form. Once again, the diastereomeric mixture was used as such without separation of the isomers. Treatment under the conditions shown gave the required substrate **11.3** in 41% yield.

Scheme 11



From this point the synthesis proceeded (see Scheme 12) in essentially the same manner as was described above for the formal synthesis of (±)-A58365A. The desired target (**6.6**) was eventually obtained and, on the basis of optical rotation measurements, was found to have been generated in approximately 92% ee. Hence, the present synthetic sequence amounts to a formal synthesis of (–)-A58365A by convergence with the Danishefsky route, but is marred by significant epimerization at the stereogenic center derived from (*S*)-(+)-2-pyrrolidinemethanol (**11.1**).

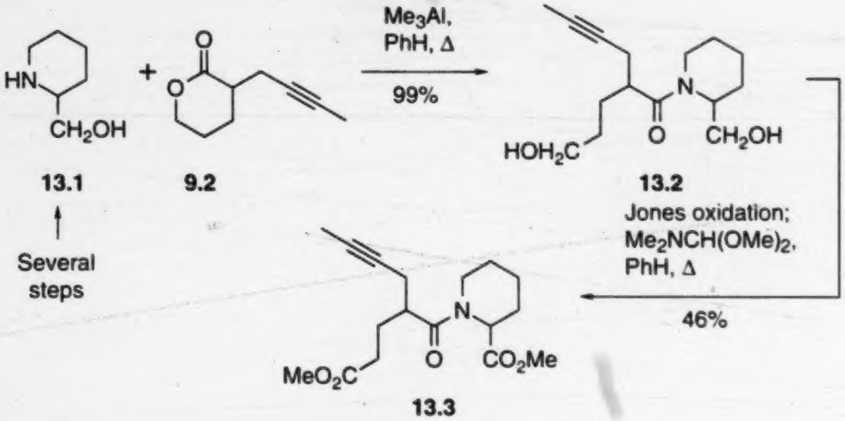
Scheme 12


carbon anode, Pt cathode, 0.03 M Et4NOTs,
MeOH, undivided cell, 36.0 mA, 6.5 F/mole; 83%
11.3
12.1
MeO
TiCl4, CH2Cl2, -78 °C to r.t.
Cl
Me
12.2
O3, MeOH, -78 °C; Zn,
AcOH, -78 °C to r.t.; 72% from 12.1
12.3
TIPSOTf, Et3N, PhH, 0 °C; 76%
OTIPS
12.4
DDQ, dioxane, Δ
51%
12.5
HCl, MeOH, H2O
48%
OH
6.6
N
O
CO2Me
MeO2C

(c) *Total Synthesis of (±)-A58365B*

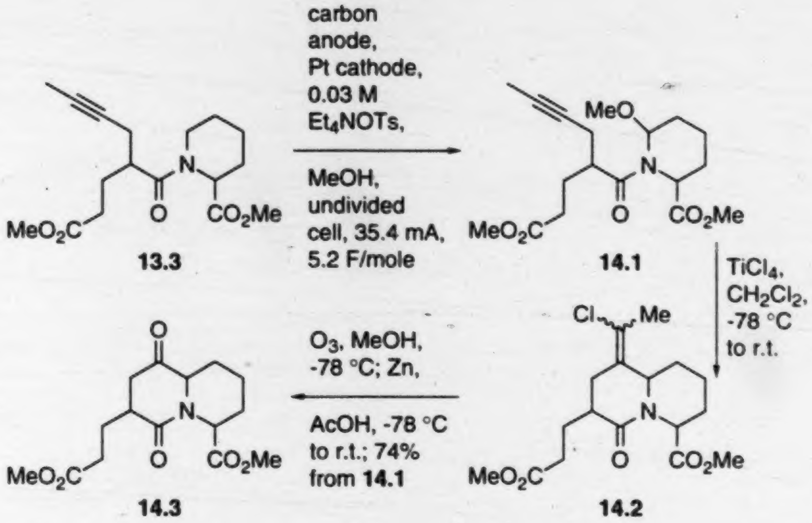
The route used in the formal synthesis of (–)-A58365A was applied, in a modified form, to the synthesis of (±)-A58365B. The electrolysis substrate in this case (**13.3**, Scheme 13) was obtained in a manner identical to that used for the preparation of **11.3** (Scheme 11) described above, with the exception that the amine used was racemic piperidinemethanol (**13.1**), rather than (*S*)-(+)-2-pyrrolidinemethanol (**11.1**).

Scheme 13



The anodic amide oxidation was carried out on the stereoisomeric mixture **13.3**, as shown in Scheme 14. The product (**14.1**) of this reaction could not be separated from unreacted starting material (**13.3**), and so the titanium-induced cyclization was carried out on the crude material. The crude product of the cyclization (**14.2**) was, in turn, used in the following ozonolysis procedure (**14.2** → **14.3**). In this way, **14.3** was obtained from **13.3** in 74% yield over three steps. Compound **14.3** was obtained as a mixture of three diastereomers (and the corresponding enantiomers). One of the diastereomers (the less polar one) could be separated from the others. Hence, the remaining transformations (see later, Scheme 15) were done in two batches. At this point, the authors were forced to make a significant departure form the synthetic route that had previously afforded both (±)-A58365A and (–)-A58365A. This departure was due to the fact that silyl enol ether

Scheme 14

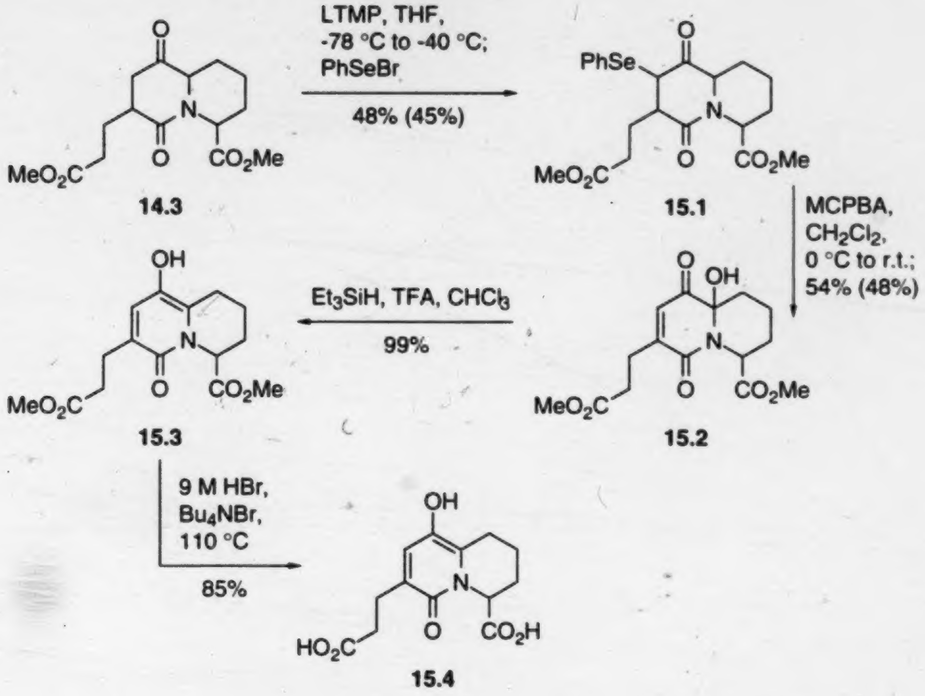


formation from **14.3** could not be effected, as had been possible using related compounds (*cf.* **7.1** → **7.3** Scheme 10, and **12.3** → **12.4** Scheme 12).

In order to complete the synthesis of (±)-A58365B, **14.3** was treated with lithium 2,2,6,6-tetramethylpiperidide, and the resulting enolate was quenched with benzeneselenenyl bromide, forming the selenated product **15.1** (Scheme 15; Yields without parentheses correspond to the faster-eluting isomer; yields in brackets correspond to the slower-eluting isomer mixture). Treatment of **15.1** with MCPBA led to the formation of **15.2**, and reduction using triethylsilane and TFA afforded compound **15.3**, which was converted into the desired product (**15.4**) by simple hydrolysis, thus completing the first total synthesis of (±)-A58365B.

Although the above syntheses by Moeller and Wong served to demonstrate the utility of anodic amide oxidation-iminium ion cyclization for making A58365A and A58365B, the synthetic routes had some significant shortcomings. Like the Danishefsky synthesis, certain steps gave low yields, especially those aimed at effecting desaturation of ring A. Not only were the actual oxidation reactions low yielding (see **7.3** → **10.3**

Scheme 15

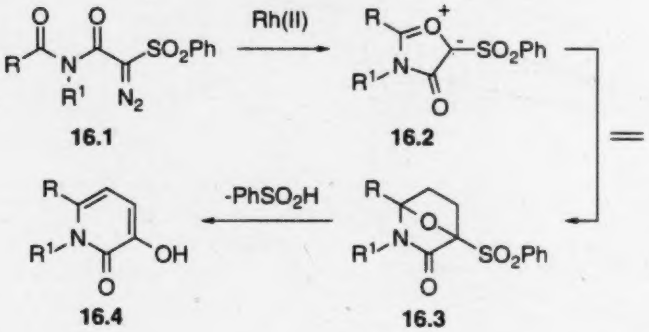


Scheme 10, **12.4** → **12.5** Scheme 12, **15.1** → **15.2** Scheme 15), but net desaturation of ring A had to be done by an indirect and inefficient route in each case (see **7.1** → **6.6** Scheme 10, **12.3** → **6.6** Scheme 12, **14.3** → **15.3** Scheme 15). Also, as was the case for the Danishefsky synthesis, no six-membered amine analog comparable to proline derivative **11.1** (Scheme 11) is readily available, and so the synthesis of A58365B must incorporate additional steps needed to make such a compound. Another shortcoming of the synthetic route of Moeller and Wong is that it was not completely general, since an alternative sequence of reactions had to be developed towards the end of the synthesis of (±)-A58365B. Finally, in the case of (–)-A58365A, the route failed to provide the product in optically pure form.

1.3.3 Formal Synthesis of (–)-A58365A by Padwa and Straub[17]

Recently, Padwa and Straub have developed an elegant and concise route to (–)-A58365A. Their synthesis incorporates, as a key step, a [3 + 2]-cycloaddition involving a phenylsulfonyl-substituted isomünchnone (*cf.* **16.2**, Scheme 16) and an appropriate dipolarophile. Once the cycloaddition has occurred (**16.2** → **16.3**), the resulting adduct undergoes spontaneous ring opening (**16.3** → **16.4**) by expulsion of $PhSO_2H$ to give the pyridone system (**16.4**).

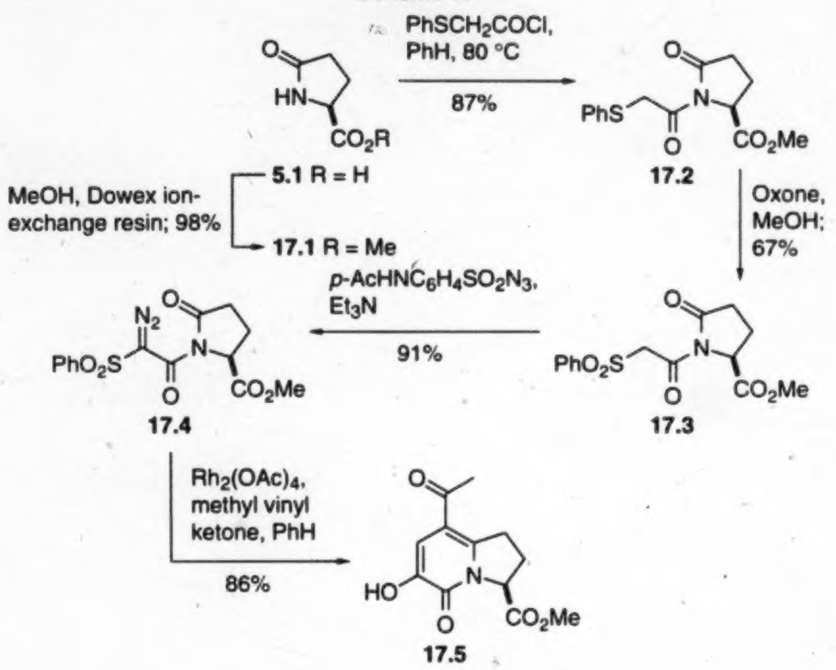
Scheme 16



The isomünchnone precursor (**17.4**) required for the synthesis of (–)-A58365A was made in four steps from L-pryroglutamic acid (**5.1**), as outlined in Scheme 17. Esterification of **5.1** with methanol in the presence of Dowex ion-exchange resin gave methyl ester **17.1**, which was subsequently converted into **17.2** by treatment with (phenylthio)acetyl chloride. Oxidation of **17.2** using Oxone served to generate sulfone **17.3**, from which diazoimide **17.4** was obtained by a standard diazotization procedure. Exposure of **17.4** to $Rh_2(OAc)_4$ in the presence of methyl vinyl ketone provided the expected pyridone (**17.5**) in good yield.

In order to incorporate the pendant propionic acid side chain of the natural product, the hydroxyl group of **17.5** was converted into the corresponding triflate (**18.1**, Scheme 18), which could then be used in a Heck reaction with methyl acrylate (**18.1** → **18.2**).

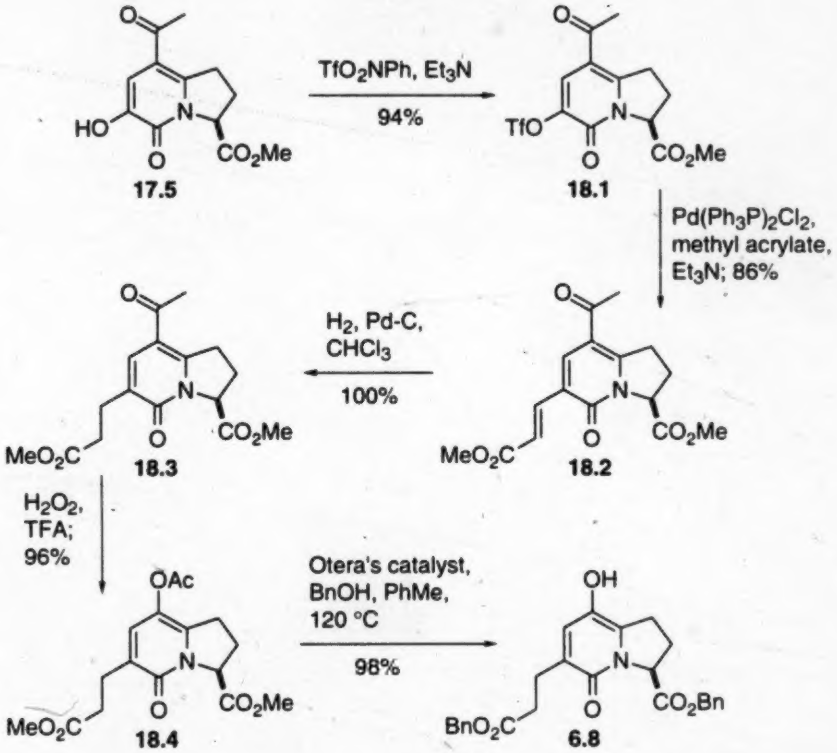
Scheme 17



The product of the Heck reaction (**18.2**) was subjected to catalytic hydrogenation, affording compound **18.3**. Baeyer-Villiger oxidation of **18.3** provided acetate **18.4** in excellent yield. All that remained at this point was simply to hydrolyze the triester to the desired product, but the authors chose instead to expose **18.4** to Otera's catalyst in the presence of BnOH, a procedure which gave compound **6.8**, and thus converged the route with that developed by Danishefsky and Fang (*cf.* Scheme 6).

The optical rotation data given for **6.8** corresponds to an ee of only 83% when compared to the value given by Danishefsky and Fang. As mentioned above, Danishefsky and Fang were able to elaborate their synthetic **6.8** to (–)-A58365A having an ee of 100%, when compared to the optical rotation of the isolated natural product, and so the ee for the synthesis described by Padwa and Straub must at best be 83%. Interestingly, the authors do not comment on this point when describing their synthesis.

Scheme 18


17.5
TfO2NPh, Et3N
94%
18.1
Pd(Ph3P)2Cl2, methyl acrylate, Et3N; 86%
18.2
H2, Pd-C, CHCl3
100%
18.3
H2O2, TFA; 96%
18.4
Otera's catalyst, BnOH, PhMe, 120 °C
98%
6.8

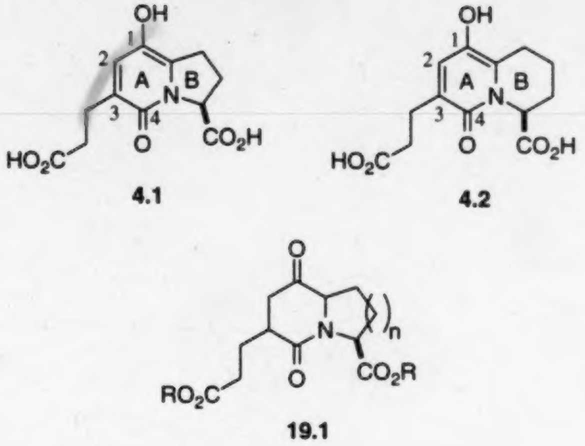
Despite the fact that their synthesis fails to provide the target compound in optically pure form, the sequence developed by Padwa and Straub is remarkably concise (11 steps) and efficient. As with the syntheses of Danishefsky and Fang, and Moeller and Wong, application of the Padwa and Straub approach to the synthesis of A58365B would require a method for preparation of a six-membered analog corresponding to pyroglutamic acid.

2 RESULTS AND DISCUSSION

2.1 *Prior Attempted Synthetic Approaches to A58365A and A58365B*

A58365A and A58365B are formally enols of α,β-unsaturated ketones. Hence, a potential synthetic approach to these compounds is to aim for the corresponding saturated materials (*cf.* **19.1**, Scheme 19), with the intention of then using one of the standard

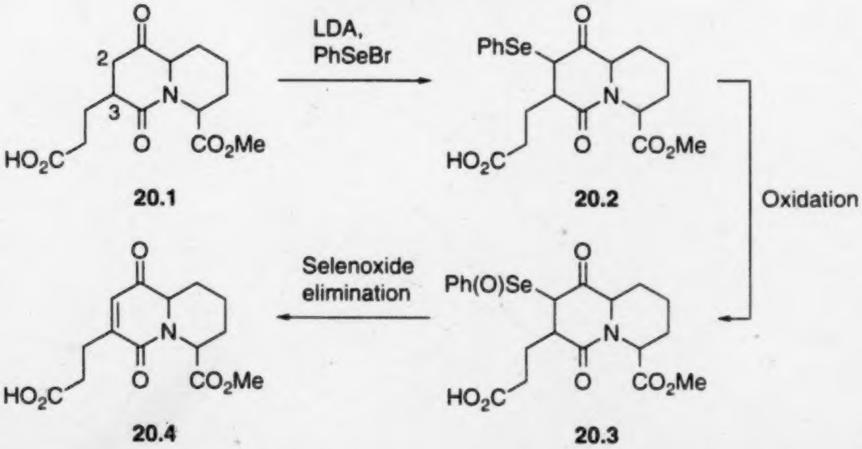
Scheme 19



methods for introducing a double bond α to a carbonyl. This is the approach that was initially taken by our group when it embarked on the total synthesis of these compounds.

In order to determine the feasibility of an approach based on compounds such as **19.1**, the bicyclic compound **20.1** was prepared[18] and its desaturation (**20.1** → **20.2** → **20.3** → **20.4**) was attempted. Unfortunately, clean oxidation of the C(2)-C(3) bond using standard selenium-based procedures could not be effected — at least not in an acceptable yield. Shortly after this approach was abandoned by our group, a similar

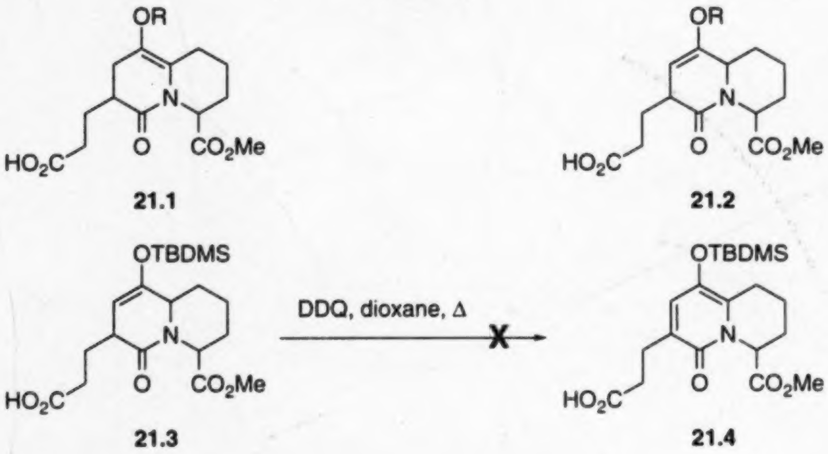
Scheme 20



observation was reported by Moeller and Wong.[16] Their efforts to introduce unsaturation **14.3** (see Scheme 15), a very similar compound to the one that had been examined by us, also proceeded extremely poorly (**14.3** → **15.1** → **15.2**). In fact, they obtained only the over-oxidized product **15.2**, which then had to be reduced (**15.2** → **15.3**).

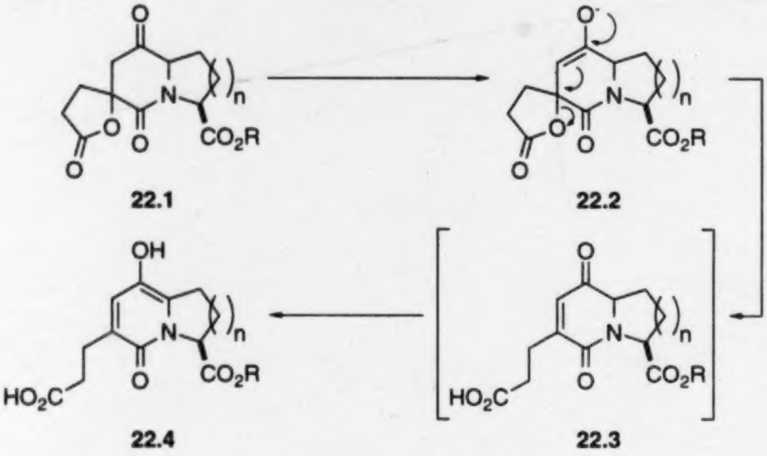
No effort was made by our group to oxidize compound **20.1**, or any corresponding enolized version of it (*cf.* **21.1** and **21.2**, Scheme 21), using DDQ, as this approach had been shown[15] by Danishefsky and Fang to be unreliable (*cf.* **6.3** → **6.4**, Scheme 6). Later, this fact was confirmed by Moeller and Wong[16] who showed that this approach either could not be implemented at all (**21.3** → **21.4**, Scheme 21), or led to over-oxidation (*cf.* **7.1** → **7.2**, and **7.3** → **7.4**, Scheme 7).

Scheme 21



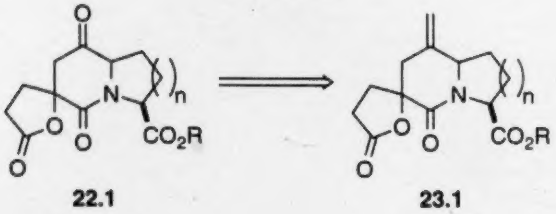
Once it had been established that late-stage introduction of the C(2)-C(3) double bond was unlikely to be useful, we decided to try a different strategy, and the approach we examined was one in which the troublesome double bond would be introduced at a much earlier stage and in the disguised form of an additional ring. We planned to prepare a spirolactone of the general type **22.1**. This type of compound has the same degree of unsaturation as the final product, and we hoped that it would be convertible into the target by treatment with mild base. The base was expected to deprotonate α to the carbonyl group, generating an enolate (**22.2**), which would then expel the lactone ring oxygen to give **22.3**. Spontaneous keto-enol tautomerization would then give a product (**22.4**) of the required structure.

Scheme 22



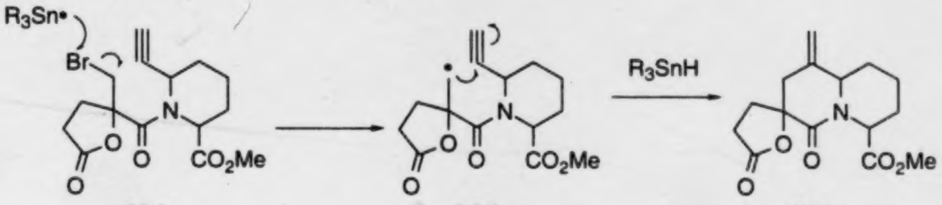
With this plan as a guide, attempts were made to prepare a suitable compound of type **22.1**. The approach that we took was based on the assumption that **22.1** could be generated by oxidative cleavage of the corresponding exocyclic olefin. In a retrosynthetic sense, this particular transform gives rise to retron **23.1** (Scheme 23), which is potentially accessible using radical cyclization-based processes. Several approaches to compounds of type **23.1**, using radical methods, were explored by our group, as described below.

Scheme 23



The first route involved acetylenic lactone **24.1**, as a key intermediate. It was hoped that **24.1** could be cyclized by a radical process along the lines shown in Scheme 24 to produce the exocyclic olefin **24.3**. Alkyl radical **24.2** would be generated by reaction of a stannyl radical with bromide **24.1**, and **24.2** could then undergo a 6-*exo digonal* ring closure to give **24.3**, after hydrogen abstraction from a stannane.

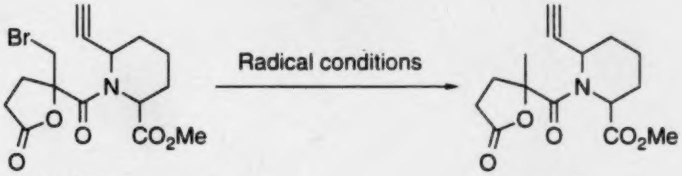
Scheme 24



24.1 **24.2** **24.3**

The acetylenic lactone **24.1** was prepared[19] but, unfortunately, radical cyclization did not proceed as expected. A variety of conditions were tried, but none of the cyclized compound **24.3** (Scheme 24) was observed, and only the corresponding reduced compound **25.1** was isolated (Scheme 25), apparently as a result of intramolecular abstraction of the propargylic hydrogen by the initially formed radical.[19]

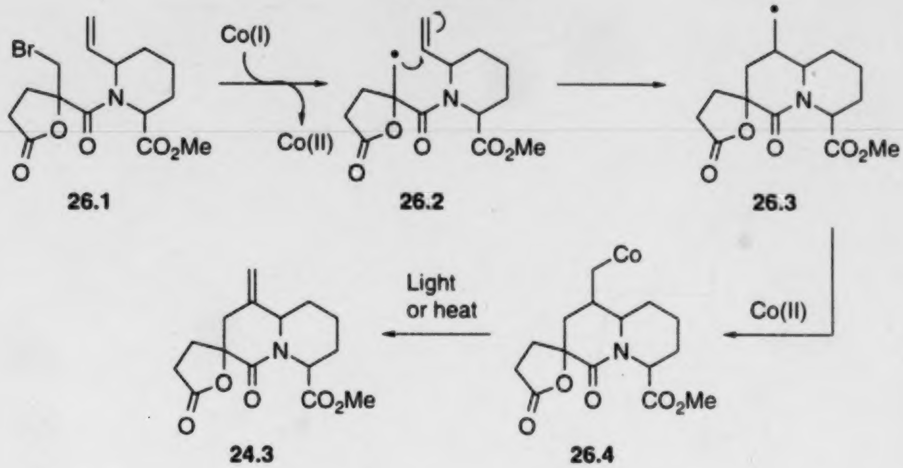
Scheme 25



24.1 **25.1**

The next approach to **22.1** was based on cobalt-mediated radical cyclization of olefinic bromide **26.1** (Scheme 26). Cobalt-mediated radical cyclization has been reported to be a powerful synthetic method for the formation of C-C bonds.[20] By analogy to the published literature, it was hoped that the reaction would proceed via electron transfer from

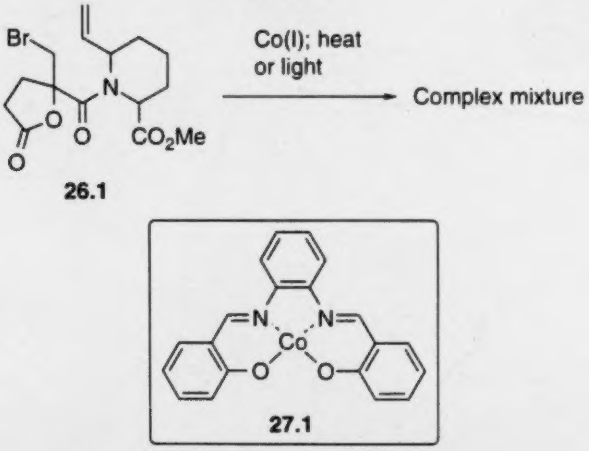
Scheme 26



a cobalt(I) species to the carbon-halogen bond of **26.1** to give **26.2**, followed by (i) intramolecular 6-*exo trigonal* cyclization (**26.2** → **26.3**), (ii) *in situ* trapping of the product radical **26.3** with Co(II) (**26.3** → **26.4**), and (iii) dehydrocobaltation (1,2-elimination) (**26.4** → **24.3**).

Once the required olefinic bromide **26.1** had been made,[19] it was treated with Co(I), generated by reduction[21] of Co(II) salophen **27.1**[22] (Scheme 27). After a reaction period of 16 hours in the dark, the intermediate was either heated or irradiated, but only

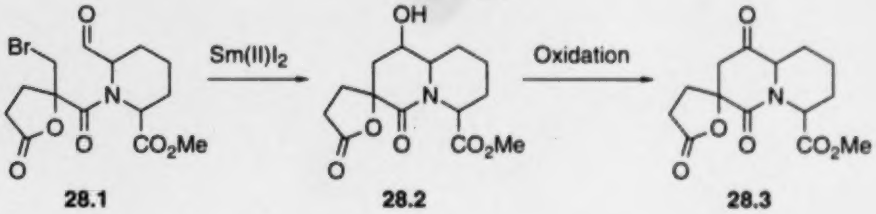
Scheme 27



complex mixtures were obtained from this experiment, with no apparent formation of the desired product, as judged by ^{1}H NMR.

A different, but related, procedure was also explored. For this strategy, aldehyde **28.1**[19] was required. Sm(II)-mediated[23] cyclization of aldehyde **28.1** to give **28.2**, followed by oxidation, could potentially lead to ketone **28.3**. However, when **28.1** was treated with SmI_2, only complex mixtures were obtained.

Scheme 28

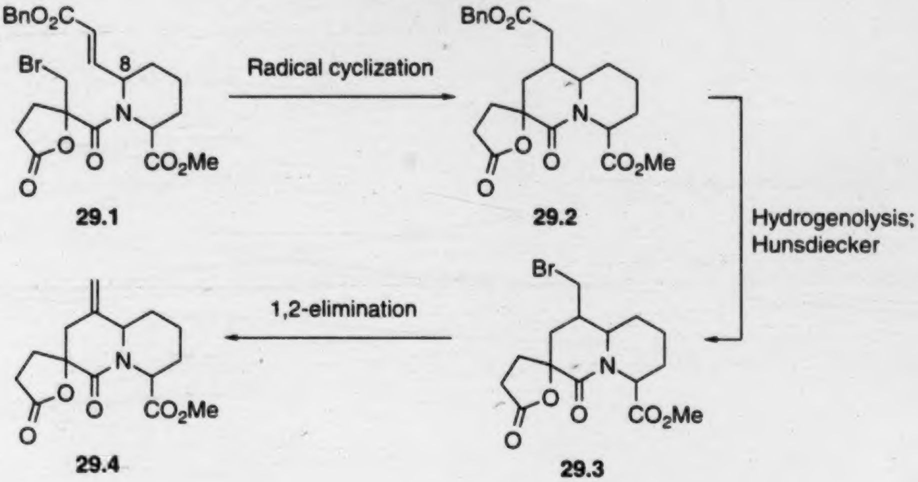


Since all attempts up to this point to effect radical cyclization on substrates having *unactivated* carbon-carbon multiple bonds had failed, cyclization of bromide **29.1** was examined. The carbon-carbon double bond in this species is in the form of an α,β-unsaturated ester and, as such, should be a more powerful radical acceptor than the parent olefin **26.1** (Scheme 26). If the bromide could be made to cyclize, then it could potentially be converted into the required exocyclic olefin by using a Hunsdiecker or related reaction, as outlined in Scheme 29.

Bromide **29.1** was prepared[19] as a mixture of diastereomers, which were separated chromatographically. Each of the diastereomers was then exposed to triphenyltin hydride and AIBN under standard thermal conditions. For one of the diastereomers, a 75% yield of the desired product (**29.2**) was obtained, but for the other diastereomer only the corresponding reduction product, presumably resulting from intramolecular hydrogen abstraction,[19] was isolated.

Evidently, the stereochemistry of the C(8) substituent was playing an important role in determining the outcome of the cyclization reaction. Notably, in the previous systems

Scheme 29



that had been examined, no cyclization could be achieved, regardless of stereochemistry at C(8). However, in the present case, an increase in the strength of the radical acceptor did allow cyclization to occur, but only in what must be the more favorable of the two cases. These observations implied two potential solutions to the problem of not being able to effect efficient radical cyclization. One of these would be to further activate the double bond to radical attack, such that cyclization from the less favorable of the two diastereomers would proceed preferentially over reduction of the initially formed radical. The second potential solution would be to make the stereochemistry of the C(8) substituent irrelevant, and it was this line of reasoning that eventually led to successful routes to A58365A and A58365B.

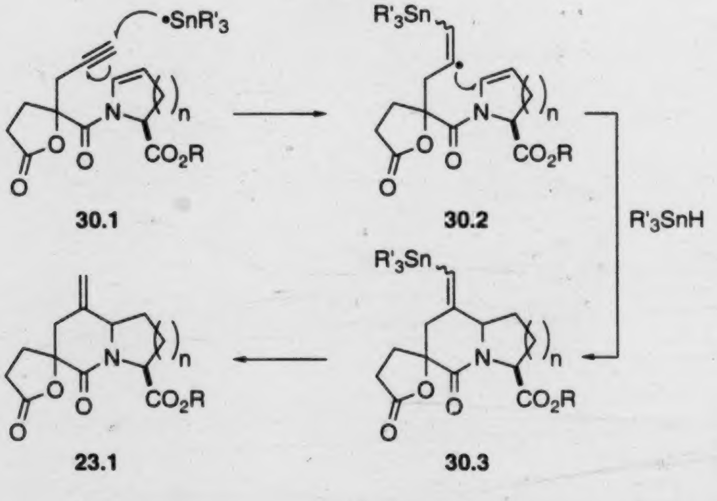
2.2 *Successful Synthetic Approach to A58365A and A58365B*

2.2.1 *The Revised Strategy*

Having recognized the importance of the stereochemistry of the C(8) substituent in influencing the outcome of the radical cyclization, we were led to modify our earlier approach and we developed a route in which the unsaturated alkyl side chain at C(8) would not be present in the radical cyclization substrate. Instead, the radical-accepting double bond would be incorporated within ring B and, in order to compensate for the change in position of the double bond, the pendant chain attached to the spirolactone would be lengthened by one carbon.

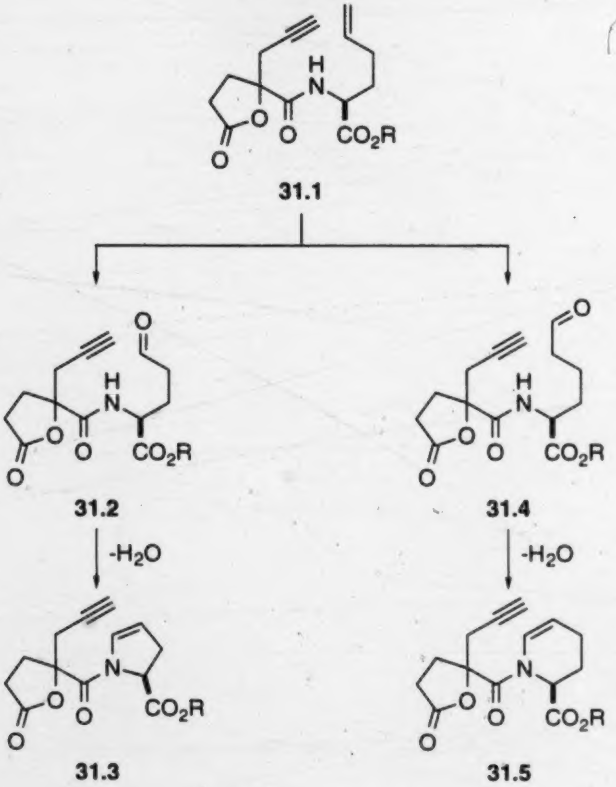
The modified plan is outlined in Scheme 30. The radical-accepting double bond is now in ring B, in the form of an enamide. Intramolecular cyclization of radicals onto the proximal terminus of an enamide double bond is known,[24] and we hoped that it would serve in the present case. As well, an acetylene unit was to be incorporated into the pendant chain of the spirolactone, as indicated in structure **30.1**. A vinyl radical would be generated by addition of a stannyl radical to the acetylene (**30.1** → **30.2**), which should then be able to cyclize onto the enamide double bond (**30.2** → **30.3**) to give vinyl stannane **30.3**. Protodestannylation (**30.3** → **23.1**) should then generate the required exocyclic olefin **23.1**.

Scheme 30



Aside from the potential of this route to simplify stereochemical matters, another particularly attractive feature was that it could also allow the formation of either A58365A, or A58365B from a common, advanced intermediate (*cf.* **31.1**, Scheme 31). This results from the fact that the radical cyclization substrates are cyclic enamides (**31.3** and **31.5**), that should be accessible by dehydration of the corresponding open-chain amido aldehydes (**31.2** and **31.4**). Each of the amido aldehydes should, in turn, be obtainable from the same advanced intermediate (**31.1**). In order to generate the five-membered enamide needed to make A58365A, the olefin would be oxidatively cleaved (**31.1** → **31.2**) to give amido aldehyde **31.2**. For the synthesis of A58365B, a six-membered enamide is required, and so the entire carbon skeleton of **31.1** would be maintained, and we would have to selectively oxidize the terminal olefinic carbon atom (**31.1** → **31.4**).

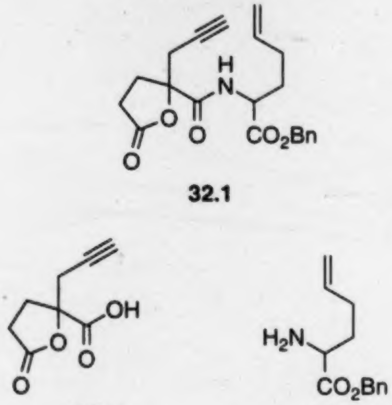
Scheme 31



2.2.2 *Synthesis of (±)-A58365A and (±)-A58365B*

We began investigating this route with a view to first synthesizing (±)-A58365A and (±)-A58365B. Hence, the advanced intermediate we decided to prepare was racemic acetylenic olefin **32.1** (Scheme 32). This compound was obtained by coupling racemic lactone acid **32.2** and racemic amino acid benzyl ester **32.3**.

Scheme 32



32.1

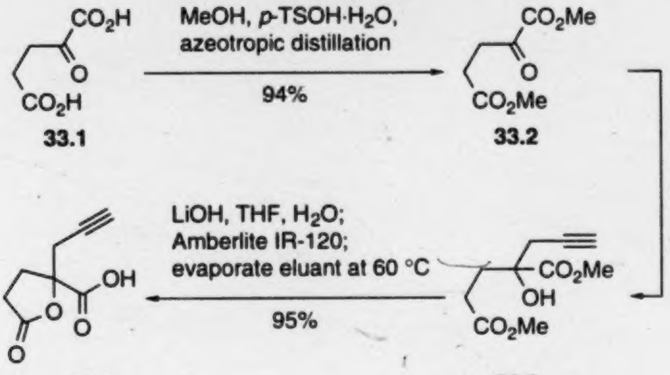
32.2 **32.3**

(a) *Preparation of the Advanced Intermediate for the Synthesis of (±)-A58365A and (±)-A58365B*

Lactone acid **32.2** was prepared from α-ketoglutaric acid (**33.1**, Scheme 33) by methylation (**33.1** → **33.2**) and treatment with the reagent[25] generated from propargyl bromide and amalgamated aluminum. In this second step (**33.2** → **33.3**) no allene was detected,[26] and the required tertiary alcohol could be isolated in high (95%) yield. Conversion into lactone acid **32.2** was achieved (95%) by base hydrolysis (LiOH), followed by ion-exchange chromatography (Amberlite IR-120), and evaporation of the eluant at 60 °C. We assume that the intermediate hydroxy diacid cyclizes during evaporation of the solvent.

The other component required for the formation of acetylenic olefin **32.1**, racemic amino acid benzyl ester **32.3**, was a known[27] compound in the optically active form and,

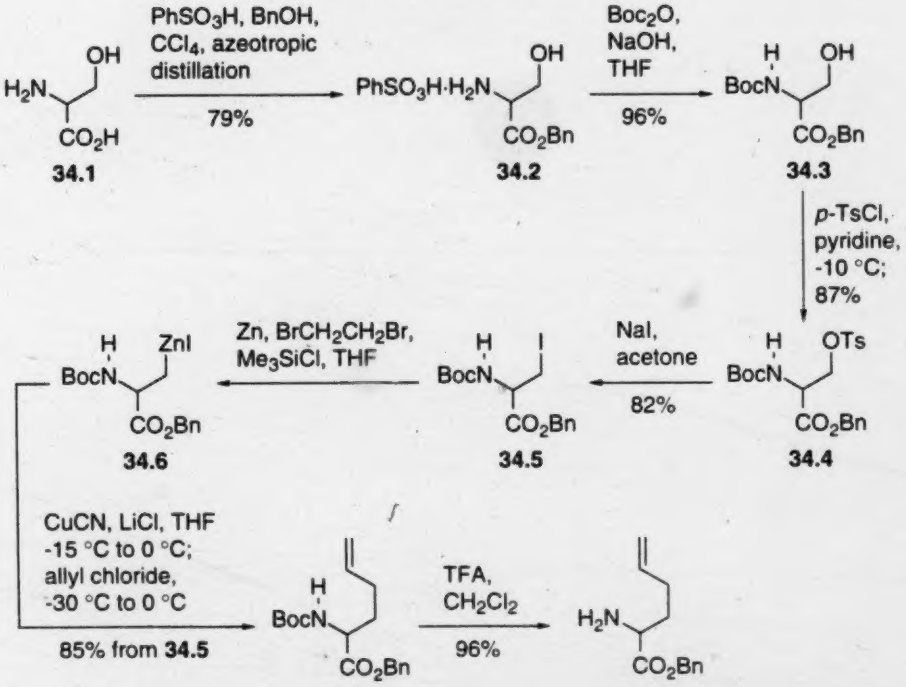
Scheme 33



32.2 **33.3**

by analogy to the synthesis of the optically active species, the racemic version was made as shown in Scheme 34. (±)-Serine (**34.1**) was esterified to give benzenesulfonate salt **34.2**, which was then treated with Boc$_2$O to give the *N*-Boc protected amino acid benzyl

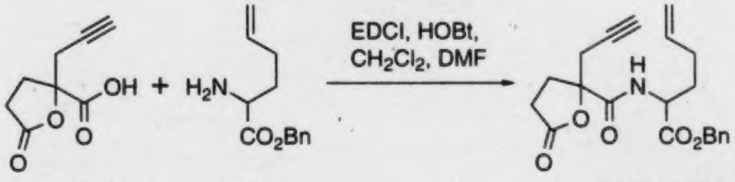
Scheme 34



ester **34.3**. The hydroxyl group of this species was activated by conversion into sulfonate **34.4**, and was then displaced with sodium iodide to form compound **34.5**. Despite the fact that we were following a literature procedure described for the synthesis of the corresponding optically active material, we found that considerable experimentation was needed in order to develop a reliable and efficient method for making the intermediate organozinc (**34.6**) from this iodide. This was eventually accomplished by a judicious blend of two literature procedures[28,29] giving the organozinc, which was subsequently transmetallated and treated with allyl chloride, forming the homoallyl glycine derivative **34.7**. TFA-mediated deprotection of the nitrogen atom of this species gave the required amino acid benzyl ester **32.3**.

Compounds **32.2** and **32.3** were coupled under standard conditions [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole; 94%] to obtain an approximately 2:1 mixture of diastereomers **32.1a,b**, which could be separated chromatographically (Scheme 35).

Scheme 35



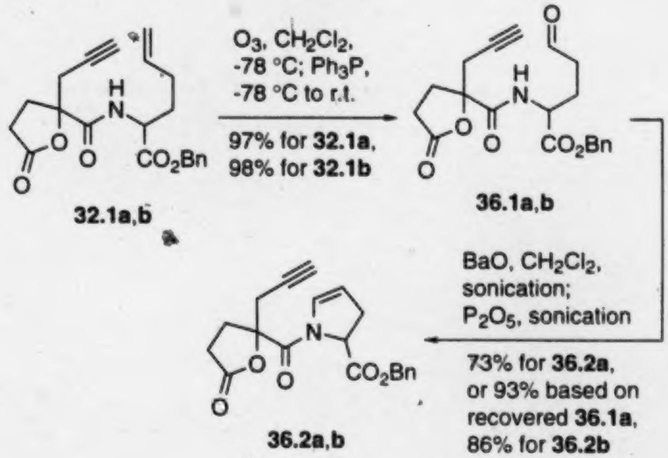
32.2 **32.3** **32.1a** (60%) **32.1b** (34%)

(b) Elaboration of the Racemic Advanced Intermediate to (±)-A58365A

Having the required acetylenic olefins in hand, each was ozonized[30] to the corresponding aldehyde (**36.1a,b**, Scheme 36) in very high yield. The next step was initially troublesome, but cyclization to enamides **36.2a,b**, was eventually achieved by sonication of the amido aldehydes in the presence of BaO, followed by addition of P_2O_5[31] (with continued sonication). This led to smooth ring closure and dehydration (**36.1a** →

36.2a, 73% or 93% based on recovered starting material; **36.1b** → **36.2b**, 86%). Both **36.2a** and **36.2b** exist as a mixture of rotamers, as judged by variable temperature ^1H NMR measurements.

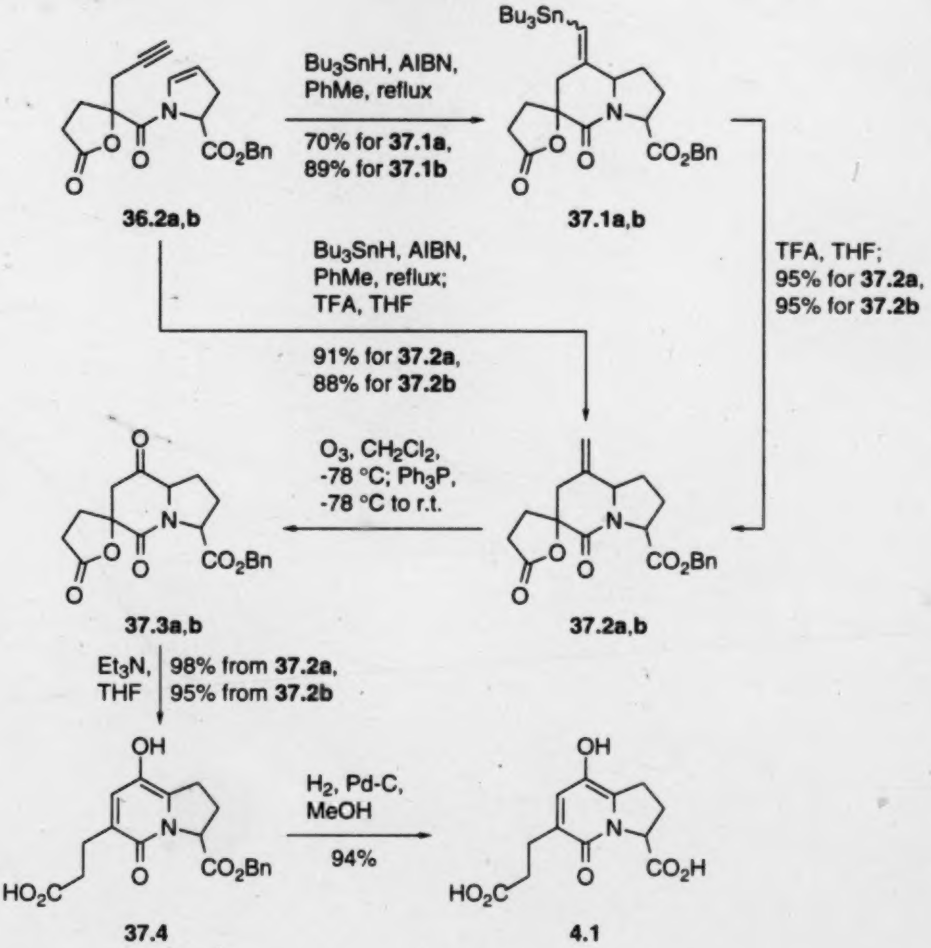
Scheme 36



O$_3$, CH$_2$Cl$_2$, -78 °C; Ph$_3$P, -78 °C to r.t.
97% for **32.1a**, 98% for **32.1b**
CO$_2$Bn
32.1a,b
36.1a,b
BaO, CH$_2$Cl$_2$, sonication; P$_2$O$_5$, sonication
73% for **36.2a**, or 93% based on recovered **36.1a**, 86% for **36.2b**
36.2a,b

Rapid addition (over a few sec) of a PhMe solution (best added in two equal portions) of Bu$_3$SnH and AIBN to a refluxing solution of **36.2a** in the same solvent gave **37.1a** (70%) after a reflux period of *ca*. 5 h (Scheme 37). Similarly, **36.2b** gave **37.1b** (89%). However, as described below, the efficiency of the process is even greater than these yields would imply. Each vinyl stannane was a single compound of undetermined double bond geometry. Although the vinyl stannanes could be purified by flash chromatography, and then subjected to protodestannylation with TFA (**37.1a** → **37.2a**; **37.1b** → **37.2b**), it was more efficient to use the crude material directly for protodestannylation. In this manner, **36.2a** was converted into **37.2a** (91% from **36.2a**), and **36.2b** into **37.2b** (88%). Ozonolytic cleavage of the exocyclic double bond of each stereoisomer proceeded efficiently (**37.2a** → **37.3a**; **37.2b** → **37.3b**), but the ketones were difficult to separate from Ph$_3$PO, formed during reductive workup of the ozonolysis mixture. Therefore, the crude ketones were treated directly with Et$_3$N at 60 °C,

Scheme 37


Bu$_3$SnH, AIBN, PhMe, reflux
70% for **37.1a**, 89% for **37.1b**
36.2a,b
Bu$_3$Sn
37.1a,b
Bu$_3$SnH, AIBN, PhMe, reflux; TFA, THF
91% for **37.2a**, 88% for **37.2b**
TFA, THF; 95% for **37.2a**, 95% for **37.2b**
O$_3$, CH$_2$Cl$_2$, -78 °C; Ph$_3$P, -78 °C to r.t.
37.3a,b
37.2a,b
Et$_3$N, THF
98% from **37.2a**, 95% from **37.2b**
H$_2$, Pd-C, MeOH
94%
37.4
4.1
CO$_2$Bn
CO$_2$H
HO$_2$C
OH
N
O

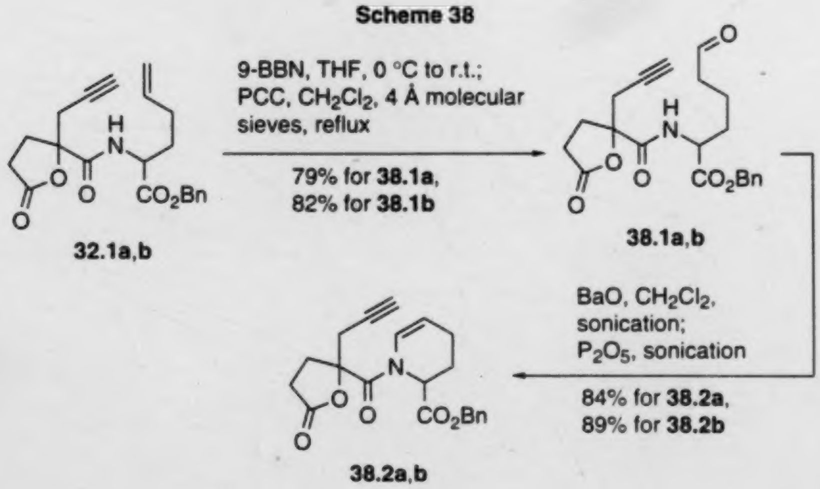
and this operation served to open the lactone, introduce the C(2)-C(3) double bond, and release the propionic acid side chain (**37.2a** → **37.4**, 98%; **37.2b** → **37.4**, 95%). Finally, hydrogenolysis of the benzyl group gave (±)-A58365A (**4.1**) in 94% yield as a pale yellow, pure, crystalline solid. Recrystallization from water produced clumps of very thin plates, but these did not diffract adequately for X-ray analysis.

(c) *Elaboration of the Racemic Advanced Intermediate to (±)-A58365B*

We next applied our synthetic route, in a slightly modified form, to the synthesis of the [4.4.0] bicycle (±)-A58365B.

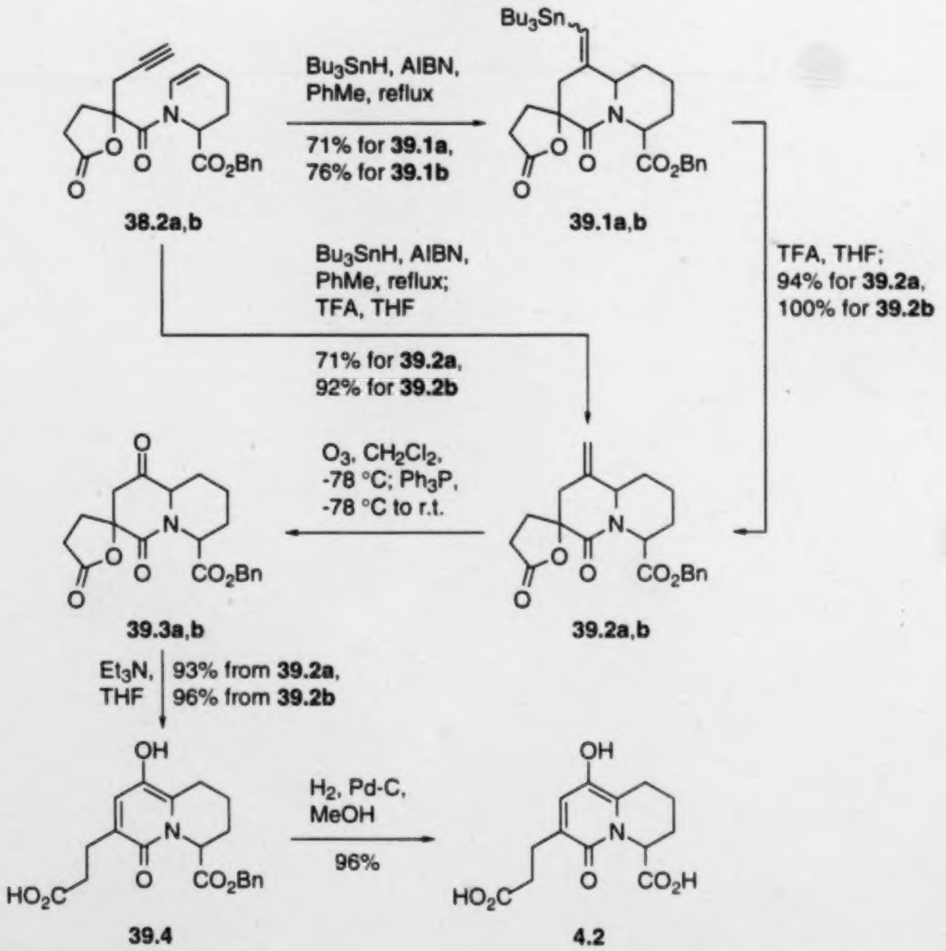
In the synthesis of (±)-A58365A, described above, the olefinic carbon chain of **32.1a,b** was shortened by oxidative cleavage of the double bond. In the present case, oxidation of the double bond is again required, but with retention of the complete carbon chain, so that a six-membered ring can eventually be generated. This oxidation was effected (Scheme 38) by taking advantage of the selectivity of 9-BBN for an alkene over an alkyne.[32] Whereas the use of 9-BBN did indeed serve to functionalize the terminal double bond, we found it difficult to isolate the derived alcohols because of hydrolysis of the benzyl ester during the borane oxidation step. We could, however, obtain the corresponding aldehydes directly, by oxidizing the intermediate boranes *in situ* with PCC[33] (**32.1a** → **38.1a**, 79%; **32.1b** → **38.1b**, 82%),[34] and so this temporary problem was actually an advantage, as we would otherwise have had to oxidize the alcohols in a separate step. From **38.1a,b**, cyclization with the BaO/P_2O_5 combination again worked well and took the route as far as enamides **38.2a,b** (84% for **38.2a**; 89% for **38.2b**).

Scheme 38



With the individual diastereomers **38.2a** and **38.2b** in hand, treatment with Bu_3SnH/AIBN — this time added in one portion — gave vinyl stannanes **39.1a,b** (Scheme 39) which were subsequently treated with TFA (**39.1a** → **39.2a**, **39.1b** → **39.2b**) to effect protodestannylation, giving exocyclic olefins **39.2a,b**. As was the case for the synthesis of (±)-A58365A, the exocyclic olefins could also be obtained without isolation of the intermediate vinyl stannanes. One of the diastereomers of **38.2a,b** exists as a mixture of rotamers (*cf.* **36.2a,b**), each of which is observable by ^{1}H NMR measurements, and this rotamer cyclizes less efficiently than the other (71% overall yield of

Scheme 39



the protodestannylated product versus 92%). In an effort to raise this yield, the radical cyclization was carried out in refluxing xylene, but we found no improvement.

The exocyclic olefins **39.2a,b** were individually ozonized and treated with Et_3N, so as to generate benzyl ester **39.4** (93% from **39.2a**; 96% from **39.2b**), and final deprotection was again done by hydrogenolysis (**39.2** → **4.2**; 96%), thus completing the synthesis of (±)-A58365B.

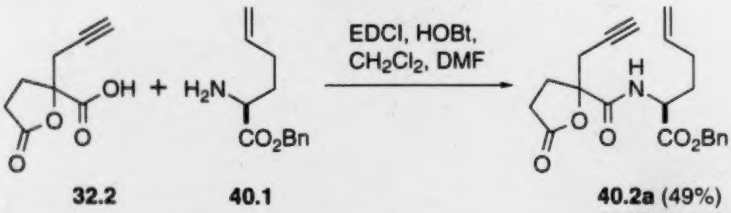
2.2.3 *Total Synthesis of (–)-A58365A and (–)-A58365B*

Having achieved the synthesis of both (±)-A58365A and (±)-A58365B, we next turned our attention to the synthesis of the optically pure natural products. It was perfectly reasonable to assume that we would be able to conduct a similar set of experiments, but beginning with optically pure serine; however, it was by no means clear whether or not the stereochemical integrity of the starting material could be maintained throughout the synthesis. Prior attempts at synthesizing these compounds in optically pure form had been marred by either partial,[16,17] or complete[16] epimerization at some stages of the work.

(a) Preparation of the Advanced Intermediate for the Synthesis of (–)-A58365A and (–)-A58365B

The advanced intermediate required for the synthesis of (–)-A58365A and (–)-A58365B was prepared by coupling our lactone acid **32.2** with *optically pure* amino acid benzyl ester **40.1** (Scheme 40).

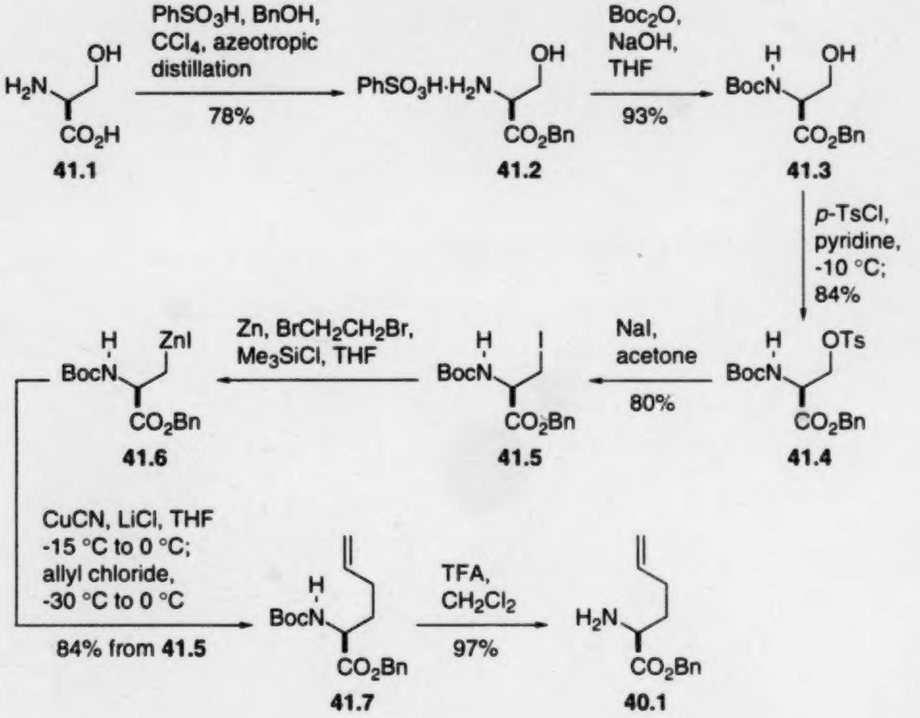
Scheme 40



40.2a (49%)
40.2b (46%)

Compound **40.1** was a known[27] substance and was prepared as outlined in Scheme 41. Hence, L-serine was converted by esterification and nitrogen protection into compound **41.3**, which was then transformed into iodide **41.5** via tosylate **41.4**. We again applied our modified procedure for preparing the organozinc compound (**41.6**), and were then able to obtain the allylated species **41.7** in good yield, from which **40.1** was generated by treatment with TFA.

Scheme 41



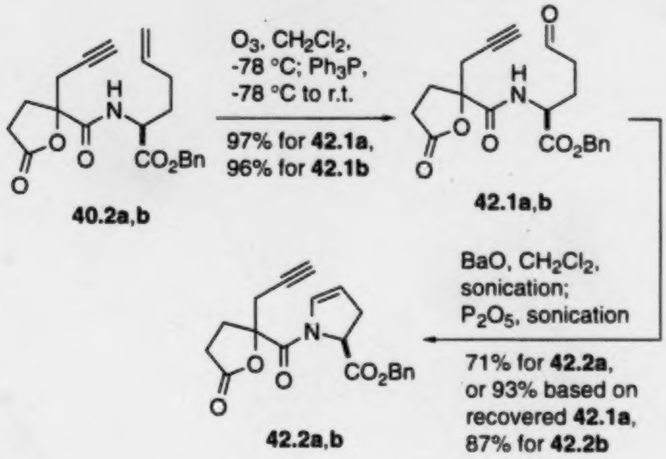
PhSO3H, BnOH, CCl4, azeotropic distillation
78%
Boc2O, NaOH, THF
93%
p-TsCl, pyridine, -10 °C; 84%
NaI, acetone
80%
Zn, BrCH2CH2Br, Me3SiCl, THF
CuCN, LiCl, THF -15 °C to 0 °C; allyl chloride, -30 °C to 0 °C
84% from 41.5
TFA, CH2Cl2
97%
41.1
41.2
41.3
41.4
41.5
41.6
41.7
40.1

(b) *Elaboration of the Optically Active Advanced Intermediate to (–)-A58365A*

Coupling of **40.1** with **32.2** gave, as already mentioned, the optically active advanced intermediate **40.2a,b** and, with this material in hand, we began our approach to (–)-A58365A along similar lines to those we had used with the corresponding racemic material.

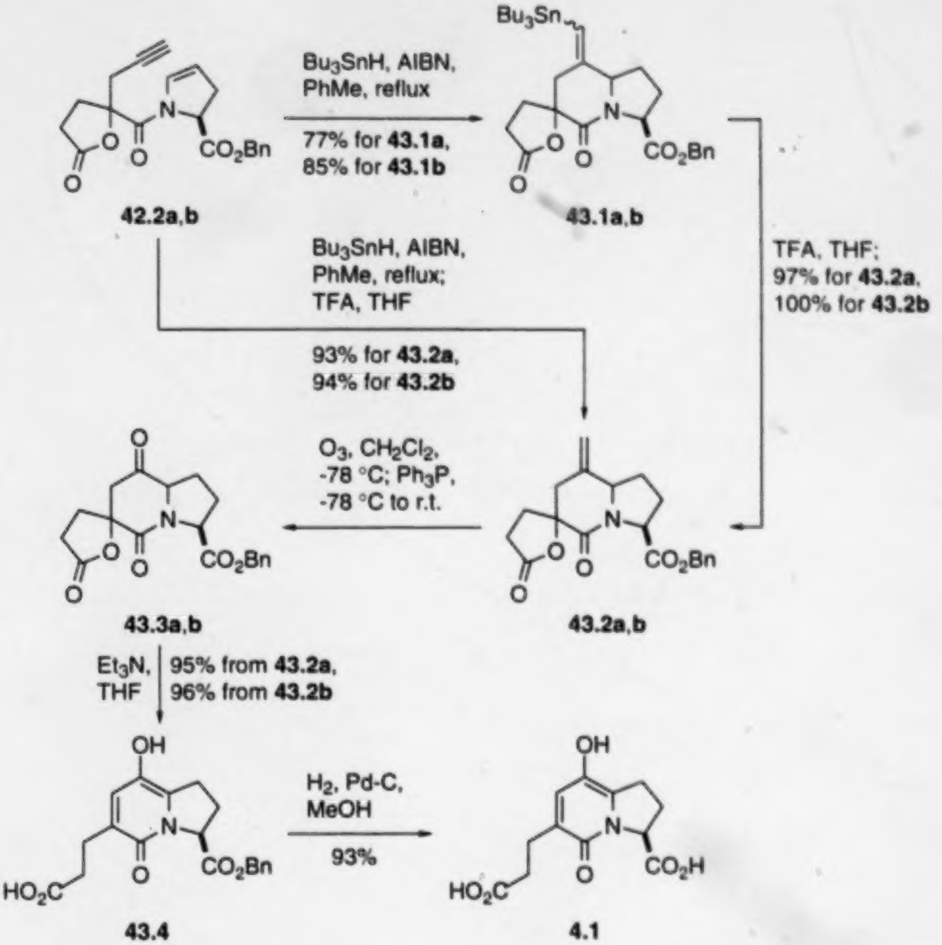
Generation of the enamides required for the radical cyclization was achieved as indicated in Scheme 42. Ozonolytic cleavage of the terminal double bond again proceeded smoothly to give the amido aldehydes **42.1a,b**, and these were then cyclized under the dehydrating conditions shown, forming the radical cyclization substrates **42.2a,b**.

Scheme 42


40.2a,b
O_3, CH_2Cl_2, -78 °C; Ph_3P, -78 °C to r.t.
97% for **42.1a**, 96% for **42.1b**
42.1a,b
BaO, CH_2Cl_2, sonication; P_2O_5, sonication
71% for **42.2a**, or 93% based on recovered **42.1a**, 87% for **42.2b**
42.2a,b
CO_2Bn

Radical cyclization from this point proceed in essentially the same manner as before (**42.2a,b** → **43.2a,b**, Scheme 43) and, once again, was carried out both with and without isolation of the intermediate vinyl stannanes (**43.1a,b**), forming exocyclic olefins **43.2a,b** in good to very good yield, depending on the specific procedure chosen. Oxidative cleavage of the olefin (**43.2a,b** → **43.3a,b**), followed by opening of the lactone ring by treatment with Et_3N (**43.3a,b** → **43.4**) reliably delivered half ester **43.4**, from which optically active (–)-A58365A was obtained as a white foam by simple hydrogenolysis. The optical rotation of our synthetic material was $[\alpha]^{25}_{D} = -196.55°$ (*c* 0.87, H_2O) which corresponds to an optical purity of 98.5%.[35] The optical purity of this material was later confirmed in a more accurate manner (see Section 2.2.3d).

Scheme 43

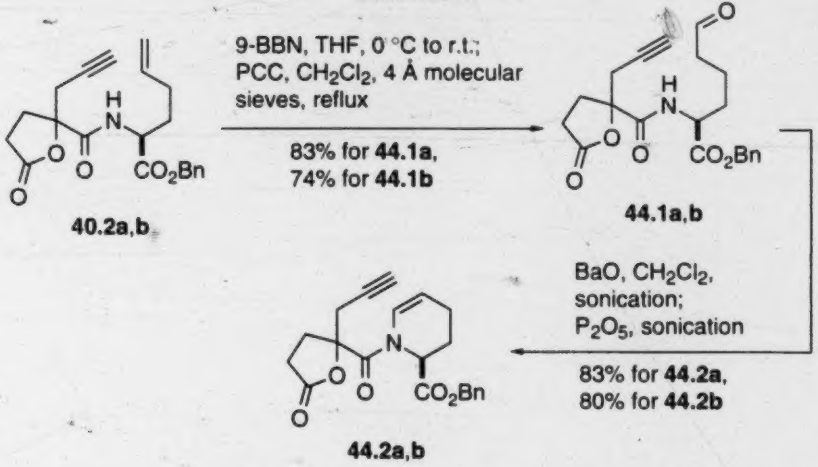


(c) Elaboration of the Optically Active Advanced Intermediate to (–)-A58365B

By this point, we were reasonably confident that we would be able to apply the synthetic approach to (±)-A58365B also to the synthesis of the corresponding optically active material. Of course, this would only be the case if the steps that had previously been used to transform **32.1a,b** into **38.1a,b** (Scheme 38) proceeded without any racemization.

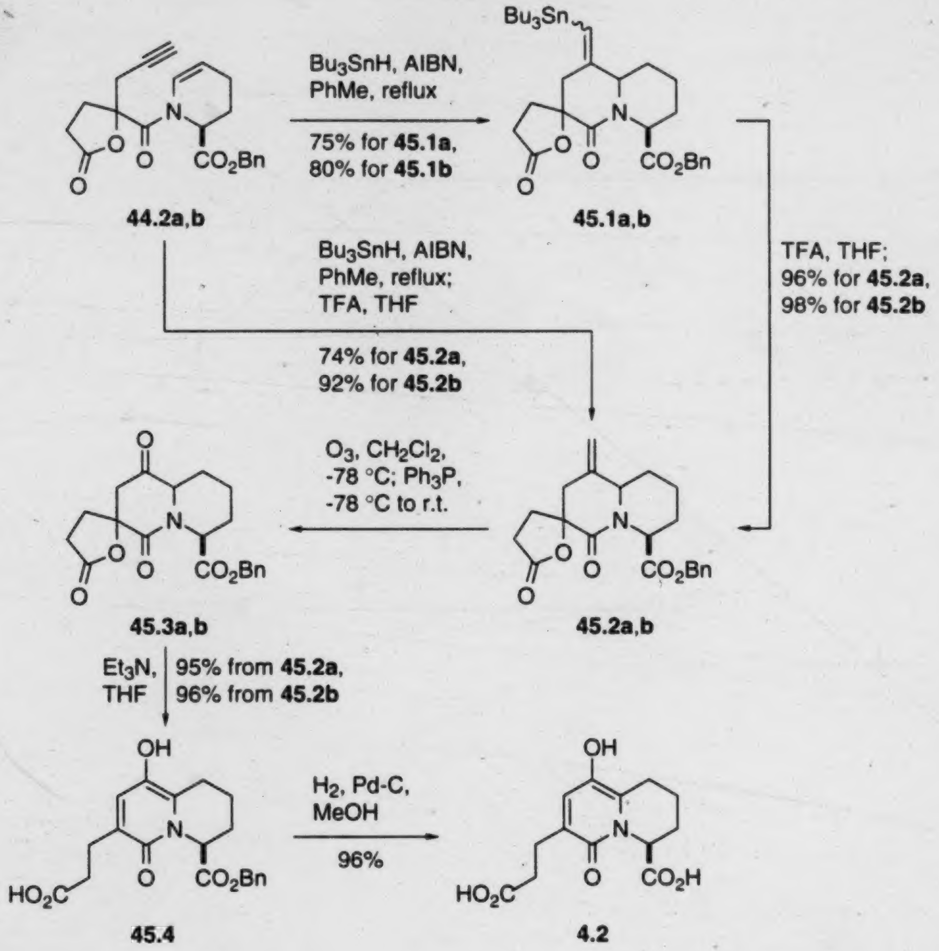
The first key transformation was carried out on the optically active advanced intermediate **40.2a,b**, as outlined in Scheme 44, and produced amido aldehydes **44.1a,b** in good yield. These were then transformed into cyclic enamides **44.2a,b** by treatment with BaO/P_2O_5.

Scheme 44



The final stages of the synthesis of (–)-A58365B commenced with the radical cyclization of enamides **44.2a,b** which gave, following protodestannylation of the derived vinyl stannanes, exocyclic olefins **45.2a,b** (Scheme 45). Oxidative cleavage of **45.2a,b** gave ketones **45.3a,b** which were treated directly with Et_3N to afforded **45.4**. Final deprotection was again done by hydrogenolysis (**45.4** → **4.2**), thus completing the first synthesis of (–)-A58365B, with 99% ee, as determined by optical rotation measurements.[36]

Scheme 45

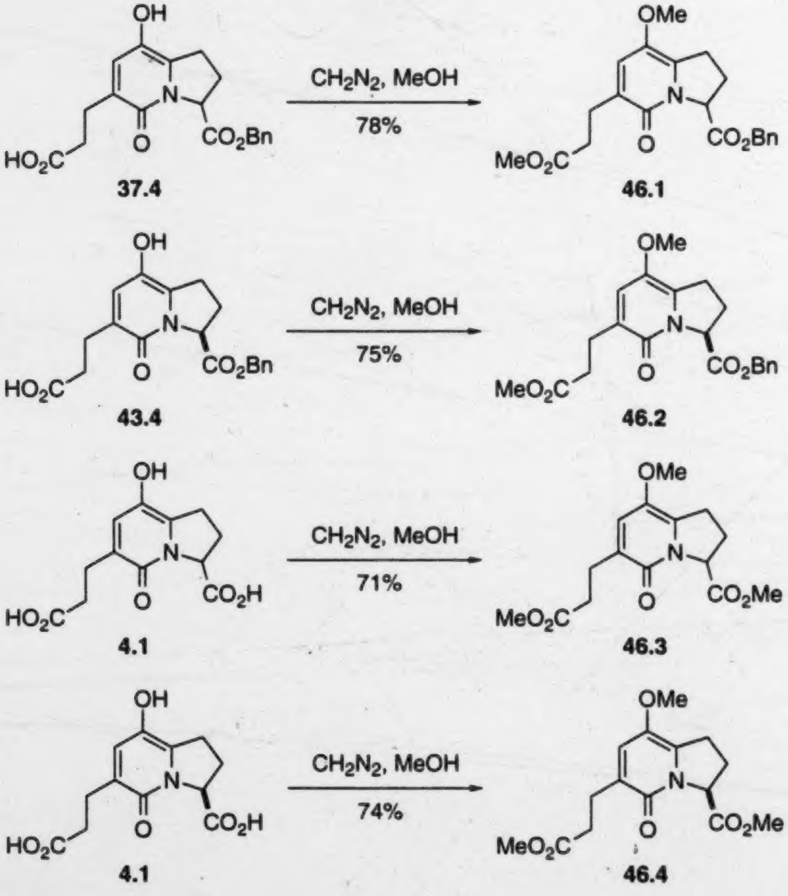

Bu$_3$SnH, AIBN, PhMe, reflux
75% for 45.1a, 80% for 45.1b
44.2a,b
45.1a,b
Bu$_3$Sn
CO$_2$Bn
TFA, THF; 96% for 45.2a, 98% for 45.2b
Bu$_3$SnH, AIBN, PhMe, reflux; TFA, THF
74% for 45.2a, 92% for 45.2b
O$_3$, CH$_2$Cl$_2$, -78 °C; Ph$_3$P, -78 °C to r.t.
45.3a,b
45.2a,b
Et$_3$N, THF
95% from 45.2a, 96% from 45.2b
H$_2$, Pd-C, MeOH
96%
OH
HO$_2$C
CO$_2$H
45.4
4.2

(d) Determination of the Enantiomeric Purity of Synthetic (–)-A58365A and (–)-A58365B

On the basis of the optical rotation data, our synthetic material appeared to be of very high optical purity. Although optical rotation measurements have historically been used as a means of determining optical purity, they are, in fact, not the most accurate measurements for this purpose. Hence, we sought to determine the enantiomeric purity of our compounds in a more precise manner, and we used the technique of high performance liquid chromatography, with a chiral, nonracemic stationary phase.[37] This method is very reliable for determining enantiomeric purity,[38] provided the individual enantiomers can be separated with baseline resolution

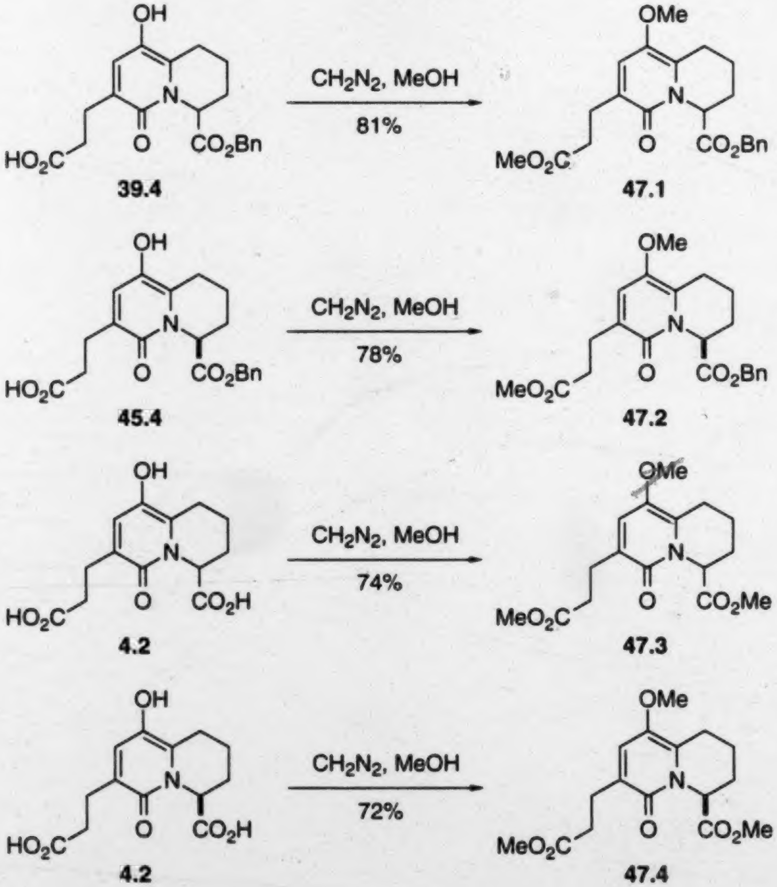
Due to the high polarity of the natural products, they were not convenient candidates for HPLC using one of the standard, commercially available, chiral, nonracemic stationary phases. As a result, we prepared permethylated derivatives of both the racemic and optically active natural products we had synthesized, as well as of their immediate synthetic precursors. In each case this was achieved by treatment of the respective hydroxyacids with an excess of diazomethane in MeOH (Scheme 46 and 47).

Scheme 46



Analysis[37] of the permethylated form (**46.2**) of optically active **43.4**, under conditions that gave baseline resolution of the enantiomers of the corresponding racemic material (**46.1**), indicated that it had an ee of 99.5%. Similar analysis of the permethylated material (**46.4**) derived from our synthetic (–)-A58365A revealed an ee of 96.2%, but in this case clear baseline resolution of the enantiomers of **46.3** could not be obtained. Analysis of our synthetically derived (–)-A58365B, and its immediate synthetic precursor **45.4**, was conducted in a similar manner and gave an ee of 99.6% for **47.2** and of 98.3% for **47.4**. The ee of our L-serine was 97%, and hence it is safe to conclude that, within the limits of experimental error, our synthetic routes involve no loss of optical integrity.

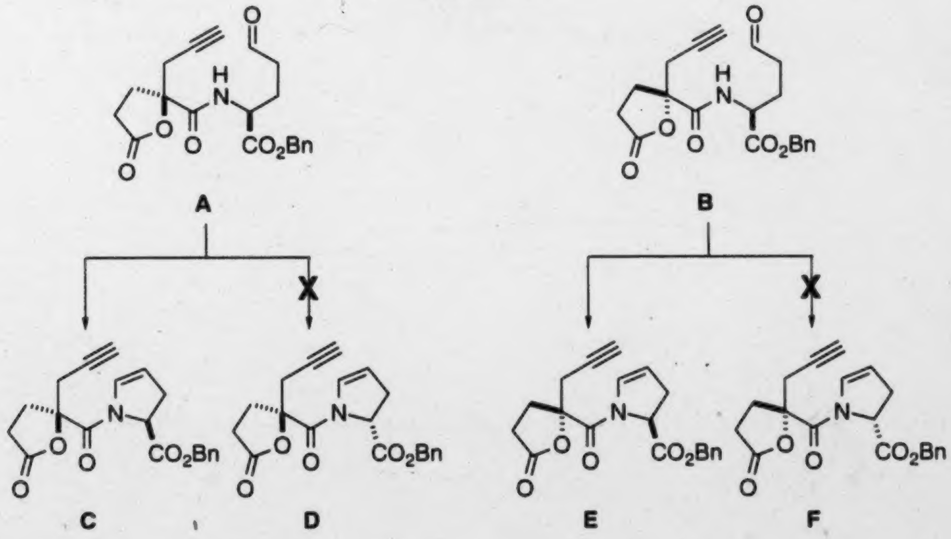
Scheme 47



Although it was gratifying to confirm by HPLC that our synthetic material was of high optical purity, we suspected well into the synthesis that the various steps were proceeding without significant epimerization at the stereogenic center derived from L-serine. This resulted from the fact that our synthetic route happened to posses convenient feature that serves as a check at each step for evidence of epimerization. This check can be explained by the example of Scheme 48.

Consider diastereomers A and B. The desired dehydration products from A and B are C and E, respectively. If epimerization at the serine-derived stereogenic center occurs in the transformation of A, then D would be formed. C and D are diastereomers and their presence together in a mixture should be detectable by NMR. Additionally, since compound D is the enantiomer of the desired product (E) derived from B, it should be identifiable by direct comparison with the NMR data for E. Since no product with an NMR spectrum like that of E was detected in the reaction mixture derived from A, we could conclude that little, if any, racemization had occurred in the reaction of A (and, of course, in the corresponding reaction of B).

Scheme 48



3 CONCLUSION

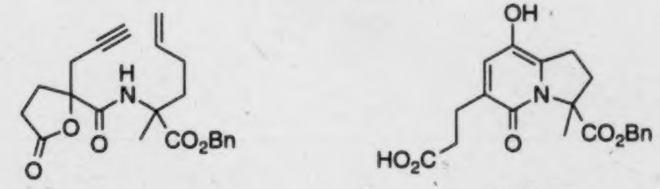
As a result of the extensive exploratory work that had been conducted in this laboratory,[18,19] we were able to devise a synthesis of both (–)-A58365A and (–)-A58365B, which has been demonstrated to deliver these compounds efficiently, reliably, and in optically pure form. Our synthetic route was based on three key transformations, each of which has been shown to work very well. These transformations are: (i) elaboration of the advanced intermediate **40.2a,b** to both amido aldehydes **42.1a,b** and **44.1a,b**, thereby allowing access to both the five- and six-membered radical cyclization substrates from a single compound, (ii) 6-*exo digonal* radical cyclization of a vinyl radical, derived by stannyl addition to an acetylene, onto the proximal position of a cyclic enamide (*cf.* **42.2a,b** → **43.1a,b**, Scheme 43) and, (iii) use of a spirolactone as a masked olefin to allow for the late-stage introduction of the C(2)-C(3) double bond, under conditions that would avoid over-oxidation of the products (*cf.* **43.3a,b** → **43.4**, Scheme 43).

4 FUTURE RESEARCH

The synthetic route that we developed for the preparation of (–)-A58365A and (–)-A58365B was devised in such a way that it would be general and, therefore, could be extended to the preparation of structurally related analogs, in a relatively straightforward way. The ability to prepare analogs of (–)-A58365A and (–)-A58365B would be extremely beneficial if one were to initiate future structure-activity relationship studies, in an effort to develop inhibitors possessing even better properties than the parent compounds. One such class of analogs that we are interested in, are those in which the amino acid derived carbon is quaternary, rather than tertiary. These compounds could potentially be more resistant to enzymatic degradation *in vivo* and, as a result, may exhibit a better pharmacological profile in terms a lower dosage requirement. Of course, these compounds would only be useful for the treatment of high blood pressure if they retain their ability to inhibit angiotensin-converting enzyme.

As a preliminary test of this, a synthesis of the methyl substituted version of A58365A (see **49.2**, Scheme 49) was carried out by another member of our group. The compound was prepared in racemic form from the advanced intermediate **49.1**, using a synthetic approach analogous to that which has been described above. *In vitro* evaluation of racemic **49.2** has shown that it is approximately 50% as active as the commercially available drug Captopril (**3.1**). It is possible that only one of the enantiomers of **49.2** is biologically active and, if this is the case, then the activity of the methyl-substituted version of A58365A may well be as good as that of Captopril. In order to confirm this, **49.2** will now have to be prepared in optically pure form. This will require the preparation of an optically pure version of the advanced intermediate **49.1**. Optically pure **49.2** can then be tested for its ability to inhibit angiotensin-converting enzyme. If the substance shows strong activity in this respect then, clearly, the preparation of other α-substituted analogs of A58365A will be justified, in an effort to develop a version which could potentially be examined further in an *in vivo* setting.

Scheme 49



49.1 **49.2**

5 EXPERIMENTAL

Unless stated to the contrary, the following conditions apply: Reactions were carried out under a slight static pressure of Ar or N_2 that had been purified by passage through a column (3.5 x 42 cm) of R-311 catalyst[39] and then through a similar column of Drierite. All solvents for reactions were dried, as described below. Glassware was dried in an oven for at least 3 h before use (140 °C) and either cooled in a desiccator over

Drierite, or assembled quickly, sealed with rubber septa, and allowed to cool under a slight static pressure of Ar or N_2. Reaction mixtures were stirred by Teflon-coated magnetic stirring bars.

Hexane and ethyl acetate used for chromatography were distilled before use.

Products were isolated from solution by evaporation under water aspirator vacuum at, or below, room temperature, using a rotary evaporator.

Microliter syringes were washed with water and acetone, using a suction device to draw the solvents through. Air was then drawn through for 1 min and the syringe was stored under vacuum. The solution to be dispensed was drawn up and expelled, and this operation was repeated several times before drawing up the sample to be used. Cannula transfers were always done under slight pressure (Ar or N_2), not by suction.

Commercial thin layer chromatography (TLC) plates (silica gel, Merck 60F–254) were used. Spots were detected by spraying the plate with a solution of phosphomolybdic acid,[40] followed by charring with a heat gun, or by examination under UV light. Silica gel for flash chromatography was Merck type 60 (230-400 mesh).

Dry solvents were prepared under an inert atmosphere and transferred by syringe or cannula. Dry THF, Et_2O, PhH, PhMe and dioxane were distilled from sodium and benzophenone ketyl. Dry CH_2Cl_2, Et_3N, i-Pr_2NEt and pyridine were distilled from CaH_2. Dry MeOH was distilled from $Mg(OEt)_2$. Acetone was distilled from K_2CO_3.

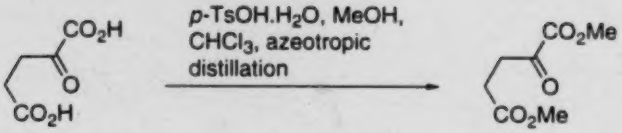
FT-IR measurements were made as casts from the specified solvent using potassium bromide plates.

The symbols s', d', t', and q' used for ^{13}C NMR signals indicate 0, 1, 2, or 3 attached hydrogens, respectively.

Mass spectra were recorded with AEI Models MS-12, MS-50, MS9 (modified), or Kratos MS50 (modified) mass spectrometers.

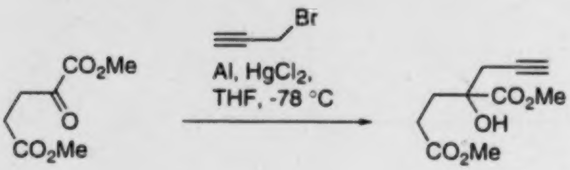
Microanalyses were performed by the microanalytical laboratory of this Department.

Dimethyl α-Ketoglutarate (33.2).[41]



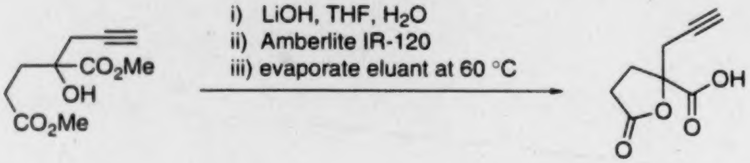
p-TsOH.H_2O (0.1005 g, 0.53 mmol), α-ketoglutaric acid (**33.1**) (5.000 g, 34.22 mmol), dry MeOH (20 mL), and dry $CHCl_3$ (40 mL) were added to a two-necked round-bottomed flask containing a magnetic stirring bar and fitted with an addition funnel containing dry $CHCl_3$ and a simple distillation head and condenser. The mixture was distilled with continuous addition of fresh $CHCl_3$ (the total volume added was *ca.* 125 mL), at a rate equal to the distillation rate, until no more water was generated (*ca.* 4.5 h). Completion of water formation was marked by an increase in the bp from 53 °C (the bp of the $CHCl_3$-water azeotrope is *ca.* 56 °C) to 59 °C (the bp of $CHCl_3$ is *ca.* 61 °C). The mixture was cooled to room temperature and the solvent was evaporated. The residue was dissolved in 60:40 EtOAc-hexanes (3 mL) and filtered through a pad (2 x 5 cm) of silica gel, using additional 60:40 EtOAc-hexanes (75 mL) as a rinse. The eluant was washed with saturated aqueous $NaHCO_3$ (25 mL), and saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated under reduced pressure (<0.1 mm Hg) to give **33.2** (5.6131 g, 94%) as a pure (^1H NMR), colorless oil: FTIR (CH_2Cl_2 cast) 2957, 2850, 1735 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.65 (t, $J = 6.2$ Hz, 2 H), 3.15 (t, $J = 6.2$ Hz, 2 H), 3.65 (s, 3 H), 3.85 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.27 (t'), 34.09 (t'), 51.85 (q'), 52.92 (q'), 160.82 (s'), 172.30 (s'), 192.16 (s'); exact mass *m/z* calcd for $C_7H_{10}O_5$ 174.0528, found 174.0525.

Dimethyl 2-Hydroxy-2-[2-(propynyl)]pentanedioate (33.3).



A mixture of Al powder[25] (84.0 mg, 3.11 mmol) and $HgCl_2$ (5 mg, 0.02 mmol) in dry THF (3 mL) was stirred vigorously for 1 h (Ar atmosphere) in a three-necked flask fitted with a reflux condenser, and closed by septa. Most of the solvent was then withdrawn by syringe from the resulting shiny Al, and fresh THF (3 mL) was injected. The mixture was warmed in an oil bath set at 40 °C, and propargyl bromide (363 mg, 3.05 mmol) in THF (1 mL) was then added slowly with vigorous stirring and at such a rate that the THF did not boil. The addition took *ca.* 10 min. After the addition, stirring at 40 °C was continued until a dark gray solution was obtained (*ca.* 1 h). This was cooled to room temperature and added by cannula at a fast dropwise rate to a stirred and cooled (-78 °C) solution of **33.2** (158 mg, 0.90 mmol) in THF (5 mL). Stirring at -78 °C was continued for 4 h, the mixture was then poured into ice-water (100 mL), and extracted with Et_2O (4 x 30 mL). The combined organic extracts were washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 16 cm), using 30:70 EtOAc-hexanes, gave pure (^{1}H NMR DMC III-101-A) **33.3** (185 mg, 95%) as a colorless oil: FTIR (KBr) 3502, 3286, 1739 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.02-2.15 [m, 3 H including a t at δ 2.05 (J = 2.7 Hz)], 2.16-2.26 (m, 1 H), 2.41-2.52 (m, 1 H), 2.56 (A of an ABX system, apparent dd, J = 16.9, 2.7 Hz, 1 H), 2.66 (B of an ABX system, apparent dd, J = 16.9, 2.7 Hz, 1 H), 3.52 (s, 1 H), 3.65 (s, 3 H), 3.78 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.65 (t'), 30.26 (t'), 32.89 (t'), 51.81 (q'), 53.26 (q'), 71.66 (s'), 75.83 (d'), 78.42 (s'), 173.36 (s'), 174.79 (s'); exact mass *m/z* calcd for $C_9H_{11}O_4$ (M - OCH_3) 183.0657, found 183.0657.

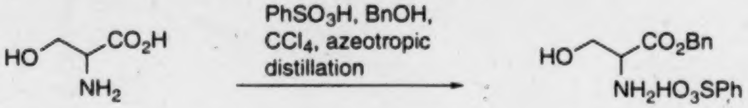
Tetrahydro-5-oxo-2-[2-(propynyl)]-2-furancarboxylic acid (32.2).



A solution of LiOH (509 mg, 10.4 mmol) in water (2 mL) was added to a solution of diester **33.3** (1.00 g, 4.67 mmol) in THF (20 mL), and the mixture was stirred overnight at room temperature. Evaporation of the solvent gave what we assume to be the dilithium salt, as a solid.

A column packed with Amberlite IR-120 ion-exchange resin (20-50 Å mesh, 2.5 x 16 cm) was washed with water until the eluant was colorless. The column was then washed successively with 2 M aqueous NaOH (4 bed volumes), water (until the eluant was neutral to pH paper), 2 M HCl (4 bed volumes), and finally with water (until the eluant was neutral to pH paper). The above dilithium salt was dissolved in water (2-3 mL) and the solution was passed down the column, using water. The eluant was monitored with pH paper or by TLC (silica gel, 10:90 MeOH-$CHCl_3$). Evaporation of the combined acidic fractions (water pump, rotary evaporator, bath temperature 65-70 °C) gave **32.2** (746 mg, 95%) as a pure (^{1}H NMR), light-yellow, powder: FTIR (KBr) 3500-2500 (br), 1770, 1720 cm^{-1}; ^{1}H NMR (D_2O, 300 MHz) δ 2.45-2.70 (m, 3 H), 2.75-2.85 (m, 2 H), 2.92 (A of an ABX system, apparent dd, *J* = 16.9, 2.9 Hz, 1 H), 3.06 (B of an ABX system, apparent dd, *J* = 16.9, 2.9 Hz, 1 H); ^{13}C NMR (D_2O, 75.5 MHz) δ 27.78 (t'), 29.03 (t'), 30.74 (t'), 73.35 (d'), 79.10 (s'), 86.90 (s'), 174.86 (s'), 180.54 (s'); exact mass *m/z* calcd for $C_5H_5O_4$ (M - $CH_2C{\equiv}CH$) 129.0188, found 129.0189; FABMS *m/z* calcd for $C_8H_8O_4$ 168.15, found 168.9.

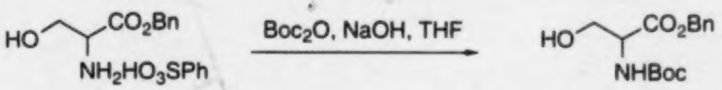
DL-Serine Benzyl Ester Benzenesulfonate (34.2).



$PhSO_3H$ (19.6533 g, 124.25 mmol), DL-serine (10.5122 g, 100.03 mmol), BnOH (50 mL, 483.17 mmol), and dry CCl_4 (125 mL) were added to a two-necked round-bottomed flask containing a magnetic stirring bar and fitted with an addition funnel containing dry CCl_4 and a simple distillation head and condenser. The mixture was distilled with continuous addition of fresh CCl_4 (the total volume added was *ca.* 250 mL), at a rate equal to the distillation rate, until no more water was generated (*ca.* 4 h). Completion of water formation was marked by an increase in the bp from 63 °C (the bp of the CCl_4-water azeotrope is *ca.* 66 °C) to 74 °C (the bp of CCl_4 is *ca.* 76 °C). The mixture was cooled to room temperature and the solvent was evaporated (<0.1 mm Hg). Et_2O (100 mL) was added to the residual viscous liquid. The resulting mixture was shaken vigorously, and then stored at -5 °C for 12 h, by which time a white precipitate had formed. The precipitate was collected, air dried, recrystallized from *i*-PrOH-Et_2O, and dried (<0.1 mm Hg) to give DL-serine benzyl ester benzenesulfonate (27.9309 g, 79%) as pure (^1H NMR), fine white crystals: mp 96-97 °C; FTIR ($CHCl_3$ cast) 3500-2600, 1607 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.85 (A of an ABX system, apparent dd, $J = 12.4, 4.6$ Hz, 1 H), 3.97 (B of an ABX system, apparent dd, $J = 12.4, 2.8$ Hz, 1 H), 4.12 (br s, 1 H), 4.80-5.30 (br s, 1 H), 4.97 and 5.02 (AB q, $\Delta\nu_{AB} = 18.0$ Hz, $J = 12.3$ Hz, 2 H), 7.14-7.31 (m, 8 H), 7.78-7.80 (m, 2 H), 8.07 (br s, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 55.51 (d'), 59.80 (t'), 68.16 (t'), 126.01 (d'), 128.21 (d'), 128.44 (d'), 128.52 (d'), 130.54 (d'), 134.59 (s'), 143.57 (s'), 167.84 (s'), two d' in the aromatic region overlap; exact mass *m/z* calcd for $C_{10}H_{14}NO_3$ (M - $C_6H_5O_3S$) 196.0974, found 196.0975; FABMS *m/z* calcd for $C_{16}H_{20}NO_6S$ (M + H) 354.09, found 354.9. Anal. Calcd for

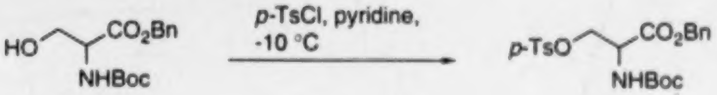
$C_{16}H_{19}NO_6S$: C 54.37, H 5.42, N 3.96, S 9.07. Found: C 54.28, H 5.35, N 3.96, S 9.13.

N-[(1,1-Dimethylethoxy)carbonyl]-DL-serine phenylmethyl ester (34.3).



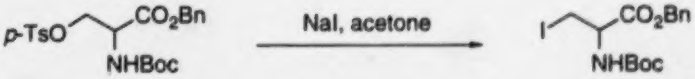
Aqueous NaOH (1 M, 100 mL) was added over *ca.* 5 min to a stirred and cooled (ice-water bath) solution of $[(t\text{-BuO})CO]_2O$ (24.24 g, 111.07 mmol) and DL-serine benzyl ester benzenesulfonate (34.50 g, 97.61 mmol) in distilled THF (200 mL). After 15 min the cooling bath was removed and stirring was continued for 3 h. The solvent was evaporated, and the residue was dissolved in EtOAc (300 mL) and acidified with 10% (w/v) aqueous $KHSO_4$. The aqueous phase was extracted with EtOAc (2 x 150 mL), and the combined organic extracts were washed with water (2 x 250 mL) and saturated aqueous NaCl (250 mL), dried ($MgSO_4$), and evaporated, to give an off-white solid. This was recrystallized from EtOAc-hexanes to afford *N*-[(1,1-dimethylethoxy)carbonyl]-DL-serine phenylmethyl ester (27.68 g, 96%) as a pure (^{1}H NMR), fine, white powder: mp 65-67 °C; FTIR ($CHCl_3$ cast) 3580-3200, 1760-1680 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H), 2.45 (br s, 1 H), 3.90 (A of an ABX system, apparent dd, J = 11.2, 3.5 Hz, 1 H), 3.98 (B of an ABX system, apparent dd, J = 11.2, 3.8 Hz, 1 H), 4.42 (br s, 1 H), 5.20 and 5.22 (AB q, $\Delta\nu_{AB}$ = 6.5 Hz, J = 12.4 Hz, 2 H), 5.52 (br d, J = 5.42 Hz, 1 H), 7.28-7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.31 (q'), 55.92 (d'), 63.55 (t'), 67.39 (t'), 80.37 (s'), 128.20 (d'), 128.49 (d'), 128.65 (d'), 135.27 (s'), 155.78 (s'), 170.74 (s'); exact mass *m/z* calcd for $C_{14}H_{19}NO_4$ (M - CH_2O) 265.1314, found 265.1316. Anal. Calcd for $C_{15}H_{21}NO_5$: C 61.00, H 7.17, N 4.74. Found: C 60.79, H 7.07, N 4.74.

N-[(1,1-Dimethylethoxy)carbonyl]-DL-serine phenylmethyl ester 4-methylbenzenesulfonate ester (34.4).



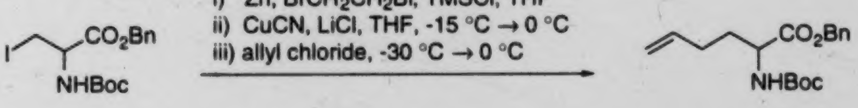
p-TsCl (0.9689 g, 5.08 mmol) was added to a stirred and cooled (-10 °C) solution of the above ester (1.3622 g, 4.61 mmol) in dry pyridine (10 mL) (Ar atmosphere). The resulting solution was stirred at -10 °C for 22 h, and then poured into ice-water (40 mL). The mixture was stirred for *ca.* 1 h, and the resulting light-pink solid was collected, washed with distilled water, and air dried for 12 h. Recrystallization from EtOH gave *N*-[(1,1-dimethylethoxy)carbonyl]-DL-serine phenylmethyl ester 4-methylbenzenesulfonate ester (1.8039, 87%) as pure (^{1}H NMR), white needles: mp 78-79 °C; FTIR (CHCl$_3$ cast) 3475-3250, 1750, 1715 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.37 (s, 9 H), 2.38 (s, 3 H), 4.30 (A of an ABX system, apparent dd, J = 10.0, 2.6 Hz, 1 H), 4.41 (B of an ABX system, apparent dd, J = 10.0, 2.5 Hz, 1 H), 4.51-4.57 (m, 1 H), 5.11 and 5.19 (AB q, $\Delta\nu_{AB}$ = 29.9 Hz, J = 12.2 Hz, 2 H), 5.41 (d, J = 8.1 Hz, 1 H), 7.26-7.37 (m, 7 H), 7.70 (d, J = 7.8 Hz, 2 H); ^{13}C NMR (CDCl$_3$, 75.5 MHz) δ 21.50 (q'), 28.09 (q'), 53.01 (d'), 67.66 (t'), 69.38 (t'), 80.29 (s'), 127.85 (d'), 128.13 (d'), 128.42 (d'), 128.50 (d'), 129.83 (d'), 132.25 (s'), 134.74 (s'), 145.02 (s'), 154.86 (s'), 168.36 (s'); exact mass *m/z* calcd for $C_{22}H_{27}NNaO_7S$ (M + Na) 472.1406, found 472.1410.

N-[(1,1-Dimethylethoxy)carbonyl]-3-iodo-DL-alanine phenylmethyl ester (34.5).



NaI (2.2524 g, 15.03 mmol) in dry acetone (14 mL) was added dropwise by cannula to a stirred solution of the above ester (4.4582 g, 9.92 mmol) in dry acetone (14 mL) (Ar atmosphere). The resulting solution was stirred in the dark for 24 h, filtered through a sintered glass frit (grade C), and evaporated. The residue was partitioned between EtOAc (75 mL) and water (50 mL), and the organic phase was washed successively with water (50 mL), aqueous $Na_2S_2O_3$ (1 M, 50 mL), water (2 x 50 mL), and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and evaporated (<0.1 mm Hg). When seeded with crystals of **34.5** (obtained by chromatography), the residue gave a yellow solid, which was recrystallized from *i*-PrOH-water to give **34.5** (3.2955 g, 82%) as pure (^{1}H NMR), white crystals: mp 52-54 °C; FTIR ($CHCl_3$ cast) 3404, 1745, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 3.56 (A of an ABX system, apparent dd, *J* = 10.3, 4.0 Hz, 1 H), 3.61 (B of an ABX system, apparent dd, *J* = 10.3, 3.7 Hz, 1 H), 4.55 (dt, *J* = 7.6, 3.8 Hz, 1 H), 5.20 and 5.23 (AB q, $\Delta\nu_{AB}$ = 13.5 Hz, *J* = 12.1 Hz, 2 H), 5.36 (d, *J* = 7.6 Hz, 1 H), 7.32-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 7.79 (t'), 28.31 (q'), 53.73 (d'), 67.98 (t'), 80.51 (s'), 128.59 (d'), 128.68 (d'), 134.89 (s'), 154.86 (s'), 169.50 (s'), two d' in the aromatic region overlap; exact mass *m/z* calcd for $C_{15}H_{20}INO_4$: 405.0437, found 405.0445.

Phenylmethyl 2-[[(1,1-Dimethylethoxy)carbonyl]amino]-5-hexenoate (34.7).[42]

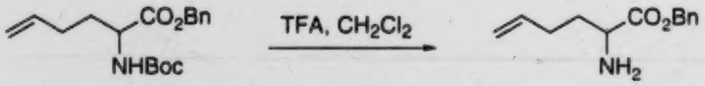

I
CO₂Bn
NHBoc
i) Zn, BrCH₂CH₂Br, TMSCl, THF
ii) CuCN, LiCl, THF, -15 °C → 0 °C
iii) allyl chloride, -30 °C → 0 °C
CO₂Bn
NHBoc

Zn dust (1.9644 g, 30.05 mmol) was added to a round-bottomed flask containing a magnetic stirring bar. The flask was sealed with a rubber septum, evacuated with an oil-pump, and refilled with Ar. The evacuation-filling process was repeated twice more, and then the system was placed under a static pressure of Ar. Dry THF (5 mL) and 1,2-dibromoethane (0.13 mL, 0.15 mmol) were added. The resulting mixture was heated to boiling with the aid of a heat gun, and then allowed to cool for 1 min. The heating-cooling process was repeated three more times, and then the flask was allowed to cool for an additional 5 min. Freshly distilled, dry Me_3SiCl (0.19 mL, 0.15 mmol) was added, and the resulting mixture was stirred for 10 min and then placed in an oil bath set at 38 °C. Stirring was continued for 10 min. A solution of **34.5** (3.0319 g, 7.48 mmol) in dry THF (12 mL) was added by cannula over *ca.* 2 min and additional THF (3 x 1.5 mL) was used as a rinse. The resulting mixture was stirred at 38 °C (Ar atmosphere) until all the starting material had been consumed (*ca.* 7 h, TLC control, silica gel, 10:90 EtOAc-hexanes).

The above solution of alkylzinc iodide **34.6** was transferred by cannula over *ca.* 3 min to a stirred and pre-cooled (bath temperature -15 °C) solution of CuCN (0.7395 g, 8.26 mmol) and LiCl (0.7098 g, 16.74 mmol) in dry THF (10 mL), and further portions of THF (2 x 2 mL) were used as a rinse to complete the transfer. The reaction flask was removed from the cold bath and placed in another at 0 °C. Stirring was continued for 15 min, and the reaction flask was then transferred to a cold bath at -30 °C. Stirring was continued for 10 min, allyl chloride (1.30 mL, 15.95 mmol) was added and, finally, the reaction flask was transferred to a cold bath at 0 °C. Stirring was continued for 12 h. The resulting solution was acidified with hydrochloric acid (2 M), and shaken with water (40 mL) and EtOAc (40 mL). The resulting white precipitate was filtered off, using a Whatman #2 filter paper, and the aqueous filtrate was extracted with EtOAc (2 x 30 mL), and filtered, as before, after each extraction to remove a small amount of precipitate. The combined organic extracts were washed with water (50 mL), filtered (as above), washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 35 cm), using 15:85 EtOAc-hexanes, gave **34.7** (2.0237 g, 85%) as a pure (^1H NMR), colorless oil: FTIR ($CHCl_3$ cast) 3470-3230, 1741, 1716 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 1.67-1.78 (m, 1 H), 1.86-1.98 (m, 1

H), 2.01-2.17 (nine-line m, 2 H), 4.30-4.40 (m, 1 H), 4.96-5.04 (m, 2 H), 5.06 (d, J = 8.0 Hz, 1 H), 5.14 and 5.20 (AB q, $\Delta\nu_{AB}$ = 25.3 Hz, J = 12.3 Hz, 2 H), 5.76 (ddt, J = 17.1, 10.3, 6.6 Hz, 1 H), 7.28-7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.32 (q'), 29.44 (t'), 31.97 (t'), 53.15 (d'), 67.00 (t'), 79.88 (s'), 115.70 (t'), 128.28 (d'), 128.42 (d'), 128.60 (d'), 135.45 (s'), 136.96 (d'), 155.32 (s'), 172.63 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{18}H_{25}NaNO_4$ (M + Na) 342.1681, found 342.1686.

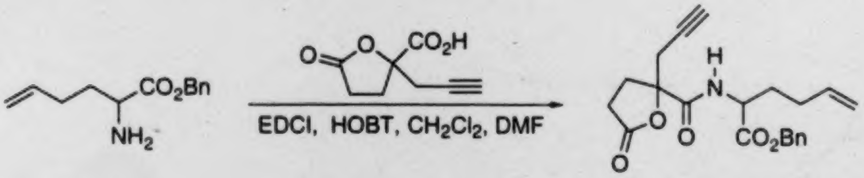
Phenylmethyl 2-Amino-5-hexenoate (32.3).



CF_3CO_2H (10 mL) was added over *ca.* 5 min to a stirred and cooled (ice-water bath) solution of **34.7** (0.727 g, 2.28 mmol) in freshly distilled CH_2Cl_2 (10 mL). The ice-water bath was removed and stirring was continued until all the starting material had been consumed (*ca.* 1 h, TLC control, silica gel, 20:80 EtOAc-hexanes), by which time the mixture had warmed to room temperature. The solution was evaporated to give a light-yellow oil, which was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ (2 x 10 mL), water (2 x 10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 90:10 EtOAc-hexanes, gave **32.3** (0.4780 g, 96%) as a pure (^{1}H NMR), colorless oil: FTIR ($CHCl_3$ cast) 3450-3250, 1735, 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (s, 2 H), 1.60-1.70 (six-line m, 1 H), 1.80-1.90 (m, 1 H), 2.10-2.18 (m, 2 H), 3.49 (dd, J = 7.8, 5.2 Hz, 1 H), 4.97 (dd, J = 10.1, 1.6 Hz, 1 H), 5.02 (ddd, J = 17.1, 3.2, 1.6 Hz, 1 H), 5.13 and 5.15 (AB q, $\Delta\nu_{AB}$ = 6.0 Hz, J = 12.4 Hz, 2 H), 5.78 (ddt, J = 17.1, 10.1, 6.6 Hz, 1 H), 7.28-7.38 (m, 5 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 29.74 (t'), 33.94 (t'), 53.91 (d'), 66.54 (t'), 115.30 (t'), 128.20 (d'), 128.28 (d'), 128.53 (d'), 135.71 (s'), 137.47 (d'), 175.83 (s'); exact mass *m/z* calcd for $C_{13}H_{17}NO_2$

219.1259, found 219.1259.

Phenylmethyl 2-[[[Tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]amino]-5-hexenoate (32.1a,b).



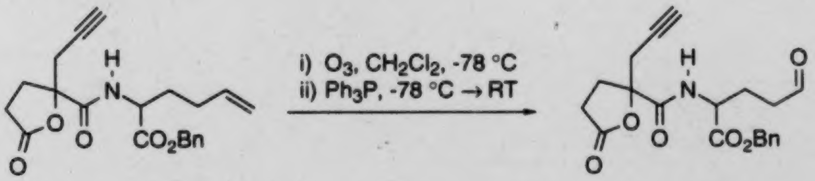
Lactone acid **32.2** (0.8742 g, 5.20 mmol), 1-hydroxybenzotriazole (0.7011 g, 5.19 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.9937 g, 5.19 mmol) were added in that order to a stirred solution of **32.3** (1.0329 g, 4.71 mmol) in a mixture of dry CH_2Cl_2 (35 mL) and freshly distilled, dry (stored over 4 Å molecular sieves) DMF (9 mL) (Ar atmosphere). Stirring at room temperature was continued for 11 h, the mixture was partitioned between $CHCl_3$ (50 mL) and water (50 mL), and the aqueous phase was extracted with $CHCl_3$ (2 x 30 mL). The combined organic extracts were washed with water (50 mL) and saturated aqueous NaCl (50 mL), dried by filtration through a pad (2 x 3 cm) of Na_2SO_4, and evaporated to give a yellow oil. Chromatography of the oil over silica gel (3 x 25 cm), repeated three times, using 3.5:3.5:3 CH_2Cl_2-Et_2O-hexanes, gave the faster-eluting diastereomer **32.1a** (1.0414 g, 60%) as a pure (^{1}H NMR), colorless oil, and the slower-eluting diastereomer **32.1b** (0.5943 g, 34%), also as a pure (^{1}H NMR), colorless oil.

Compound **32.1a** had: FTIR ($CHCl_3$ cast) 3348, 3295, 1788, 1740, 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77-1.88 (six-line m, 1 H), 1.91-2.08 (m, 4 H), 2.39-2.57 (m, 3 H), 2.64-2.82 (m, 3 H), 4.54 (dt, J = 8.1, 4.9 Hz, 1 H), 4.94-5.01 (m, 2 H), 5.12 and 5.19 (AB q, $\Delta\nu_{AB}$ = 24.0 Hz, J = 12.2 Hz, 2 H), 5.71 (ddt, J = 16.8, 10.5, 6.5 Hz, 1 H), 7.08 (d, J = 8.1 Hz, 1 H), 7.26-7.36 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz)

δ 28.33 (t', two signals overlap), 29.43 (t'), 29.56 (t'), 30.88 (t'), 52.03 (d'), 67.22 (t'), 72.39 (d'), 77.18 (s'), 85.27 (s'), 116.09 (t'), 128.32 (d'), 128.48 (d'), 128.57 (d'), 135.13 (s'), 136.49 (d'), 170.80 (s'), 170.99 (s'), 175.02 (s'); exact mass *m/z* calcd for $C_{21}H_{23}NO_5$ 369.1576, found 369.1573.

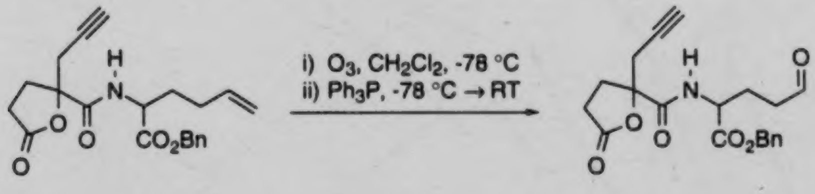
Compound **32.2b** had: FTIR ($CHCl_3$ cast) 3354, 3295, 1789, 1739, 1677 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.76-1.87 (m, 1 H), 1.96-2.14 (m, 4 H), 2.32-2.56 (m, 3 H), 2.61-2.74 (m, 1 H), 2.84 (d, *J* = 2.6 Hz, 2 H), 4.62 (dt, *J* = 8.3, 4.7 Hz, 1 H), 4.99-5.60 (m, 2 H), 5.10 and 5.19 (AB q, $\Delta\nu_{AB}$ = 37.6 Hz, *J* = 12.1 Hz, 2 H), 5.75 (ddt, *J* = 17.9, 9.8, 6.5 Hz, 1 H), 6.85 (d, *J* = 8.3 Hz, 1 H), 7.31-7.41 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.16 (t'), 28.56 (t'), 29.57 (t'), 30.01 (t'), 30.93 (t'), 52.05 (d'), 67.40 (t'), 72.32 (d'), 77.38 (s'), 85.43 (s'), 116.40 (t'), 128.45 (d'), 128.65 (d'), 128.72 (d'), 135.17 (s'), 136.44 (d'), 170.72 (s'), 171.17 (s'), 175.00 (s'); exact mass *m/z* calcd for $C_{21}H_{23}NO_5$ 369.1576, found 369.1580.

5-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-DL-norvaline phenylmethyl ester (36.1a).



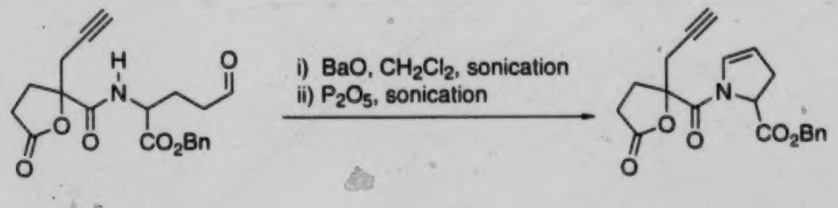
Freshly distilled CH_2Cl_2 (15 mL) was added to **32.1a** (0.9112 g, 2.47 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 10 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (1.3119 g, 5.00 mmol) was added. The cooling bath was removed and stirring was continued for 4 h, by which time the mixture had warmed to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (3 x 25 cm), using 90:10 EtOAc-hexanes, gave **36.1a** (0.8884 g, 97%) as an oil, containing trace amounts of what we take to be the corresponding *E* and *Z* enol tautomers (^1H NMR): FTIR ($CHCl_3$ cast) 3356, 3290, 2834, 2731, 1789, 1739, 1678 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.95-2.90 [m, 11 H, including a t at δ 2.02 ($J = 3.0$ Hz), and a t at δ 2.75 ($J = 3.0$ Hz)], 4.55 (dt, $J = 7.9$, 5.3 Hz, 1 H), 5.13 and 5.18 (AB q, $\Delta\nu_{AB} = 18.4$ Hz, $J = 12.1$ Hz, 2 H), 7.06 (d, $J = 7.9$ Hz, 1 H), 7.27-7.40 (m, 5 H), 9.67 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 24.11 (t'), 28.34 (t'), 28.51 (t'), 29.45 (t'), 39.87 (t'), 51.95 (d'), 67.57 (t'), 72.46 (d'), 77.17 (s'), 85.25 (s'), 128.50 (d'), 128.60 (d'), 128.70 (d'), 134.99 (s'), 170.53 (s'), 171.13 (s'), 175.16 (s'), 200.45 (d'); exact mass *m/z* calcd for $C_{20}H_{21}NO_6$ 371.1369, found 371.1371.

5-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-DL-norvaline phenylmethyl ester (36.1b).



Freshly distilled CH_2Cl_2 (10 mL) was added to **32.1b** (0.3694 g, 1.00 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 5 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.5271 g, 2.01 mmol) was added. The cooling bath was removed and stirring was continued for 4 h, by which time the mixture had warmed to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 20 cm), using 70:30 EtOAc-hexanes, gave **36.1b** (0.3638 g, 98%) as an oil, containing trace amounts of what we take to be the corresponding *E* and *Z* enol tautomers (^1H NMR): FTIR ($CHCl_3$ cast) 3356, 3287, 2834, 2732, 1789, 1739, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.97-2.12 [m, 2 H, including a t at δ 2.09 (J = 2.8 Hz)], 2.22-2.74 (m, 7 H), 2.76-2.90 [m, 2 H, including a t at δ 2.83 (J = 3.0 Hz)], 4.60 (dt, J = 8.3, 5.0 Hz, 1 H), 5.10 and 5.16 (AB q, $\Delta\nu_{AB}$ = 22.7 Hz, J = 12.1 Hz, 2 H), 7.11 (d, J = 8.3 Hz, 1 H), 7.28-7.44 (m, 5 H), 9.70 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 24.06 (t'), 28.12 (t'), 28.51 (t'), 30.02 (t'), 39.85 (t'), 51.85 (d'), 67.64 (t'), 72.33 (d'), 85.37 (s'), 128.52 (d'), 128.65 (d'), 128.75 (d'), 134.98 (s'), 170.63 (s'), 170.96 (s'), 174.99 (s'), 200.37 (d'), the internal acetylene carbon signal overlaps the solvent signals; exact mass *m/z* calcd for $C_{20}H_{21}NO_6$ 371.1369, found 371.1369.

Phenylmethyl 2,3-Dihydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-1*H*-pyrrole-2-carboxylate (36.2a).

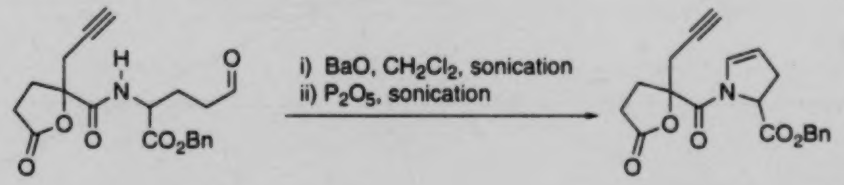


BaO (0.6833 g, 4.46 mmol) was tipped into a solution of **36.1a** (0.8029 g, 2.16 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.7977 g, 5.62 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde was being consumed (*ca.* 3 h, TLC control, silica gel, 60:40 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (2.5 x 25 cm), using 60:40 EtOAc-hexanes, gave **36.2a** (0.5575 g, 73%, 93% after correction for recovered starting material) as a pure (^{1}H NMR), colorless oil, which was a mixture of rotamers. The material should be used within 24 h. Further elution with 90:10 EtOAc-hexanes gave starting material (0.1712 g, 0.46 mmol) (^{1}H NMR) as an oil.

Compound **36.2a** had: FTIR ($CHCl_3$ cast) 3282, 1792, 1745, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.03-2.12 [m, 1 H, including a t at δ 2.10 (J = 3.0 Hz)], 2.32-3.24 [m, 8 H, including a ddt at δ 3.19 (J = 17.5, 11.5, 2.5 Hz)], 4.86-5.42 [m, 4 H, including a dd at δ 4.89 (J = 11.5, 4.2 Hz), an AB q at δ 5.07 and 5.20 ($\Delta\nu_{AB}$ = 52.9 Hz, J = 12.1 Hz), overlapping an AB q at δ 5.12 and 5.23 ($\Delta\nu_{AB}$ = 46.3 Hz, J = 12.2 Hz), overlapping a dd at δ 5.25 (J = 7.0, 2.7 Hz), overlapping a dd at δ 5.28 (J = 4.7, 2.5 Hz), and a dd at δ 5.39 (J = 11.2, 2.7 Hz)], 7.05-7.23 [m, 1 H, including a ddd at δ 7.07 (J = 4.3, 2.9, 1.6 Hz), and a dt at δ 7.21 (J = 4.4, 2.2 Hz)], 7.31-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.76 (t'), 28.11 (t'), 28.63 (t'), 29.38 (t'), 30.72 (t'), 31.25 (t'), 32.21 (t'), 36.42 (t'), 59.38 (d'), 59.76 (d'), 67.21 (t'), 67.90 (t'), 72.19 (d'), 72.81 (d'), 86.18 (s'), 86.32 (s'), 109.96 (d'), 111.36 (d'), 128.38 (d'), 128.54 (d'), 128.66 (d'), 128.71 (d'), 129.48 (d'), 131.03 (d'), 135.20 (s'), 135.36 (s'), 165.99 (s'), 167.88 (s'), 170.25 (s'), 172.42 (s'), 175.16 (s'), 175.38 (s'), two pairs of d' in the aromatic region overlap (i.e. only two signals are observed); the internal acetylene carbon signals overlap the solvent signals; exact mass *m/z* calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1265.

If the aldehyde solution is not pre-treated with BaO for *ca.* 1 h before addition of P_2O_5, the overall yield is <50%. In the absence of BaO, the P_2O_5 turns into a reddish-brown gummy material after sonication in CH_2Cl_2 for 1 h or more; when BaO is present, the P_2O_5 stays as a fine powdered suspension. If additional P_2O_5 is added to the system during the course of the reaction in an attempt to improve the conversion, an unidentified byproduct is formed, and the yield of **36.2a** is reduced.

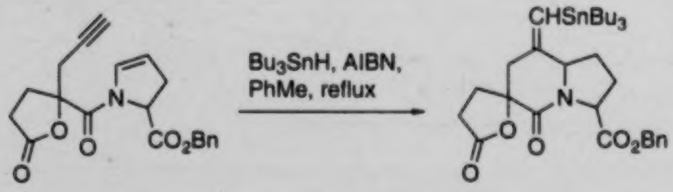
Phenylmethyl 2,3-Dihydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-1*H*-pyrrole-2-carboxylate (36.2b).



BaO (0.4326 g, 2.82 mmol) was tipped into a solution of **36.1b** (0.2686 g, 0.72 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.3977 g, 2.80 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 3 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (1.5 x 20 cm), using 50:50 EtOAc-hexanes, gave **36.2b** (0.2198 g, 86%) as a pure (^{1}H NMR), colorless oil, which was a mixture of rotamers: FTIR ($CHCl_3$ cast) 3282, 1792, 1745, 1641 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.03-2.13 [m, 1 H, including a t at δ 2.11 (*J* = 2.6 Hz)], 2.28-2.46 (m, 2 H), 2.52-2.62 (m, 2 H), 2.74-3.02 (m, 4 H), 4.97 (dd, *J* = 11.7, 5.2 Hz, 1 H), 5.04-5.29 [m, 3 H, including an AB q at δ 5.07 and 5.27 ($\Delta\nu_{AB}$ = 75.1 Hz, *J* = 12.3 Hz), and a dt at δ 5.21 (*J* = 4.6, 2.3 Hz)], 7.02-7.24 [m, 1 H, including a five-line m at δ 7.04, and a dt δ 7.21 (*J* = 4.4, 2.2 Hz)], 7.28-7.43 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.73 (t'), 28.10 (t'), 28.53 (t'), 30.89 (t'), 31.65 (t'), 31.93 (t'), 36.63 (t'), 59.43 (d'), 59.82 (d'), 67.02 (t'), 67.62 (t'), 72.17 (d'), 72.65

(d'), 76.90 (s'), 85.91 (s'), 86.24 (s'), 109.48 (d'), 111.00 (d'), 128.16 (d'), 128.28 (d'), 128.37 (d'), 128.55 (d'), 129.29 (d'), 131.02 (d'), 135.08 (s'), 135.24 (s'), 166.35 (s'), 168.89 (s'), 170.15 (s'), 171.65 (s'), 174.93 (s'), 175.24 (s'), not all signals for the minor rotamer were observed; exact mass *m/z* calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1269.

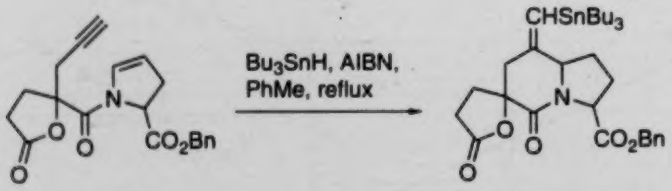
Phenylmethyl Octahydro-5,5'-dioxo-8'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylic acid (37.1a).



A solution of AIBN (0.0108 g, 0.07 mmol, 8.1 mM) and Bu_3SnH (0.25 mL, 0.93 mmol, 0.11 M) in dry PhMe (8.6 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **36.2a** (0.1527 g, 0.43 mmol) in PhMe (8.6 mL) (Ar atmosphere). Stirring at reflux was continued for 3 h. A solution of AIBN (0.0103 g, 0.06 mmol, 14.0 mM) and Bu_3SnH (0.25 mL, 0.93 mmol, 0.22 M) in dry PhMe (4.3 mL) was then injected by syringe over *ca.* 10 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.5 h, TLC control, silica gel, 40:60 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the oily residue over silica gel (2.5 x 25 cm), using 30:70 EtOAc-hexanes, gave **37.1a** (0.1921 g, 70%) as a white, amorphous solid, containing slight impurities (^{1}H NMR): FTIR ($CHCl_3$ cast) 1788, 1745, 1664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78-1.05 (m, 15 H), 1.21-1.64 (m, 13 H), 1.74-2.08 (m, 3 H), 2.14-2.29 (m, 2 H), 2.38-2.42 (m, 1 H), 2.60-2.95 (m, 4

H), 4.41-4.48 (m, 1 H), 4.55 (d, *J* = 9.7 Hz, 1 H), 5.09 and 5.23 (AB q, $\Delta\nu_{AB}$ = 54.2 Hz, *J* = 12.3 Hz, 2 H), 5.83 (vinyl proton, d, *J* = 1.7 Hz, 1 H, Sn satellite signals at δ 5.78 and 5.90), 7.23-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 10.32 (t'), 13.66 (q'), 27.27 (t'), 28.23 (t'), 28.67 (t'), 28.97 (t'), 29.97 (t'), 30.48 (t'), 44.08 (t'), 58.38 (d'), 63.79 (d'), 67.06 (t'), 83.46 (s'), 125.69 (d'), 128.17 (d'), 128.40 (d'), 128.59 (d'), 135.48 (s'), 147.09 (s'), 166.93 (s'), 171.07 (s'), 176.22 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{32}H_{47}NNaO_5{}^{120}Sn$ (M + Na) 668.2374, found 668.2376.

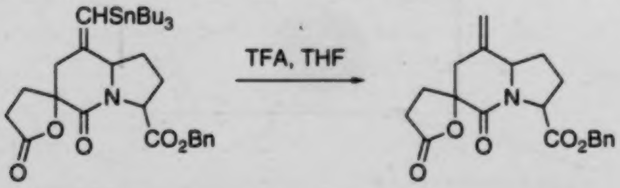
Phenylmethyl Octahydro-5,5'-dioxo-8'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylic acid (37.1b).



A solution of AIBN (0.0051 g, 0.03 mmol, 8.3 mM) and Bu_3SnH (0.10 mL, 0.37 mmol, 0.37 M) in dry PhMe (3.6 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **36.2b** (0.0641 g, 0.18 mmol) in PhMe (3.6 mL) (Ar atmosphere). Stirring at reflux was continued for 2.5 h. A solution of AIBN (0.0049 g, 0.03 mmol, 16.6 mM) and Bu_3SnH (0.10 mL, 0.37 mmol, 0.21 M) in dry PhMe (1.8 mL) was then injected by syringe over *ca.* 10 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1 h, TLC control, silica gel, 50:50 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the oily residue over silica gel (2 x 20 cm), using 35:65 EtOAc-hexanes, gave **37.1b** (0.1017 g, 89%) as a

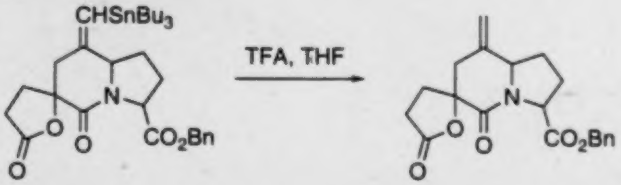
colorless oil, containing slight impurities (^{1}H NMR): FTIR (CHCl$_3$ cast) 1788, 1748, 1665 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.79-1.04 (m, 15 H), 1.21-1.83 (m, 13 H), 1.83-1.97 (m, 2 H), 2.25-2.33 (five-line m, 1 H), 2.34-2.46 (m, 1 H), 2.47-2.64 (m, 3 H), 2.80-2.94 (m, 1 H), 3.01-3.09 [m, 1 H, including a d at δ 3.05 (J = 14.4 Hz)], 4.48 (dd, J = 10.2, 4.7 Hz, 1 H), 4.56 (t, J = 8.5 Hz, 1 H), 5.14 and 5.21 (AB q, $\Delta\nu_{AB}$ = 29.7 Hz, J = 12.4 Hz, 2 H), 5.86 (vinyl proton, d, J = 1.5 Hz, 1 H, Sn satellite signals at δ 5.79 and 5.92), 7.25-7.37 (m, 5 H); ^{13}C NMR (CDCl$_3$, 50.3 MHz) δ 10.40 (t'), 13.60 (q'), 27.20 (t'), 27.45 (t'), 28.53 (t'), 29.05 (t'), 31.03 (t'), 31.61 (t'), 44.15 (t'), 58.42 (d'), 63.88 (d'), 66.94 (t'), 82.74 (s'), 127.11 (d'), 128.09 (d'), 128.25 (d'), 128.57 (d'), 135.50 (s'), 146.08 (s'), 167.09 (s'), 171.13 (s'), 175.85 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{32}H_{47}NNaO_5{}^{120}Sn$ (M + Na) 668.2374, found 668.2378.

Phenylmethyl Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (37.2a).



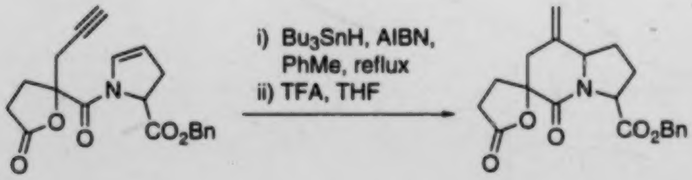
Dry CF$_3$CO$_2$H (0.5 mL) was injected rapidly into a stirred solution of **37.1a** (0.0919 g, 0.143 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 1 h no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 25 cm), using 60:40 EtOAc-hexanes, gave **37.2a** (0.0482 g, 95%) as a pure (^{1}H NMR), white crystals (mp 145-147 °C), spectroscopically identical to material obtained without isolation of the vinyl stannane.

Phenylmethyl Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (37.2b).



Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of **37.1b** (0.0794 g, 0.123 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 20 min no more vinyl stannane could be detected (TLC control, silica gel, 35:65 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **37.2b** (0.0416 g, 95%) as a pure (^1H NMR), colorless oil, spectroscopically identical to material obtained without isolation of the vinyl stannane.

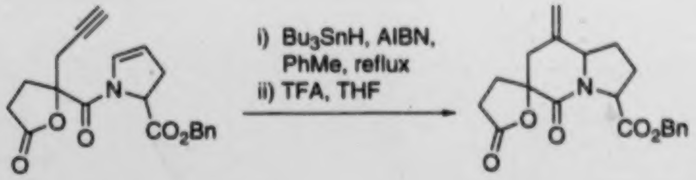
Phenylmethyl Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (37.2a) without isolation of the intermediate stannane.



A solution of AIBN (0.0331 g, 0.20 mmol, 7.7 mM) and Bu_3SnH (0.74 mL, 2.75 mmol, 0.11 M) in dry PhMe (26 mL) was injected by syringe over *ca.* 1 min into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **36.2a** (0.4639 g, 1.31 mmol) in PhMe (26 mL) (Ar atmosphere). Stirring at reflux was continued for 3 h. A solution of AIBN (0.0335 g, 0.20 mmol, 15.4 mM) and Bu_3SnH (0.74 mL, 2.75 mmol, 0.21 M) in dry PhMe (13 mL) was then injected by syringe over *ca.* 30 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.75 h, TLC control, silica gel, 40:60 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (2.0 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (25 mL) (Ar atmosphere). After *ca.* 1.5 h no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 25 cm), using 60:40 EtOAc-hexanes, gave **37.2a** (0.4246 g, 91%) as a pure (^{1}H NMR), white powder: mp 145-147 °C; FTIR ($CHCl_3$ cast) 1782, 1742, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.83-2.02 (m, 2 H), 2.04-2.12 (m, 1 H), 2.18-2.32 (m, 2 H), 2.49 (ddd, J = 17.3, 9.6, 2.9 Hz, 1 H), 2.64-2.86 [m, 3 H, including a dt at δ 2.80 (J = 17.3, 9.6 Hz)], 3.01 (dd, J = 14.9, 1.2 Hz, 1 H), 4.32-4.39 (m, 1 H), 4.54 (d, J = 8.8 Hz, 1 H), 5.01-5.03 (m, 1 H), 5.05-5.25 [m, 3 H, including an AB q at δ 5.07 and 5.21 (Δv_{AB} = 54.8 Hz, J = 12.2 Hz)], 7.28-7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.39 (t'), 28.58 (t'), 29.10 (t'), 31.01 (t'), 42.20 (t'), 58.17 (d'), 61.82 (d'), 67.16 (t'), 83.09 (s'), 112.06 (t'), 128.24 (d'), 128.47 (d'), 128.64 (d'), 135.44 (s'), 139.13 (s'), 167.32 (s'), 171.05 (s'), 176.42 (s'); exact mass *m/z* calcd for $C_{20}H_{21}NO_5$ 355.1420, found 355.1425.

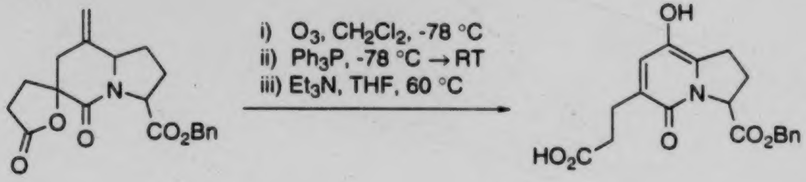
Phenylmethyl Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (37.2b) without isolation of the intermediate stannane.



A solution of AIBN (0.0029 g, 0.02 mmol, 8.7 mM) and Bu_3SnH (0.07 mL, 0.26 mmol, 0.11 M) in dry PhMe (2.3 mL) was injected by syringe over *ca.* 5 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **36.2b** (0.0398 g, 0.113 mmol) in PhMe (2.3 mL) (Ar atmosphere). Stirring at reflux was continued for 2.5 h. A solution of AIBN (0.0031 g, 0.02 mmol, 16.7 mM) and Bu_3SnH (0.07 mL, 0.26 mmol, 0.22 M) in dry PhMe (1.2 mL) was then injected by syringe over *ca.* 5 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (5 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 50:50 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1 x 20 cm), using 80:20 EtOAc-hexanes, gave **37.2b** (0.0352 g, 88%) as a pure (^1H NMR), colorless oil: FTIR ($CDCl_3$ cast) 1782, 1745, 1659 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.74 (ddt, J = 19.0, 11.8, 7.8 Hz, 1 H), 1.86-2.04 (m, 2 H), 2.21-2.33 (five-line m, 1 H), 2.39-2.58 (m, 3 H), 2.65 (d, J = 13.9 Hz, 1 H), 2.86 (dt, J = 17.7, 10.5 Hz, 1 H), 3.09 (dd, J = 13.9, 1.0 Hz, 1 H), 4.37 (dd, J = 10.9, 4.9 Hz, 1 H), 4.55 (t, J = 8.6 Hz, 1 H), 5.04 (br s, 1 H), 5.12 (br s, 1 H), 5.15 and 5.21 (AB q, $\Delta\nu_{AB}$ = 25.9 Hz, J = 12.4 Hz, 2 H), 7.25-7.38 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 27.58 (t'), 28.37 (t'), 30.11 (t'), 32.37 (t'), 42.43 (t'), 58.01 (d'), 61.94 (d'), 67.00 (t'), 82.42 (s'), 113.10 (t'), 128.07 (d'), 128.29 (d'), 128.58 (d'), 135.44 (s'), 138.18 (s'), 167.55 (s'), 171.16 (s'), 176.13 (s'); exact mass *m/z* calcd for $C_{20}H_{21}NO_5$ 355.1420, found 355.1421.

1,2,3,5-Tetrahydro-8-hydroxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoic acid (37.4) from 37.2a.

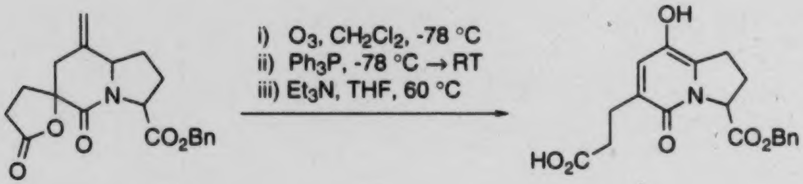


Freshly distilled CH_2Cl_2 (15 mL) was added to **37.2a** (0.1844 g, 0.52 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 8 min, TLC control, silica gel, 60:40 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.2753 g, 1.05 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product (**37.3a**) could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

Dry Et_3N (2.0 mL, 14.35 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (20 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (2.5 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **37.4** (0.1817 g, 98%) as pure (^{1}H NMR), white crystals: mp 120-122 °C; FTIR (microscope) 3375-2450, 1742, 1711, 1531 cm^{-1}; ^{1}H NMR (CD_3OD, 360 MHz) δ 2.26 (ddt, *J* = 13.4, 8.6, 3.3 Hz, 1 H), 2.49-2.63 (m, 3 H), 2.68-2.83 (eight-line m, 2 H), 2.95-3.17 (thirteen-line m, 2 H), 5.13-5.26 [m, 3 H, including a dd at δ 5.16 (*J* = 9.6, 3.2 Hz), overlapping an AB q at δ 5.12 and 5.21 ($\Delta\nu_{AB}$

= 14.1 Hz, *J* = 12.5 Hz)], 7.24 (s, 1 H), 7.27-7.38 (m, 5 H); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 26.90 (t'), 27.48 (t'), 28.09 (t'), 33.78 (t'), 63.92 (d'), 68.33 (t'), 129.19 (d'), 129.38 (d'), 129.59 (d'), 129.80 (s'), 133.96 (s'), 134.90 (d'), 136.98 (s'), 137.07 (s'), 160.37 (s'), 171.46 (s'), 176.76 (s'); exact mass *m/z* calcd for $C_{19}H_{19}NO_6$ 357.1213, found 357.1216.

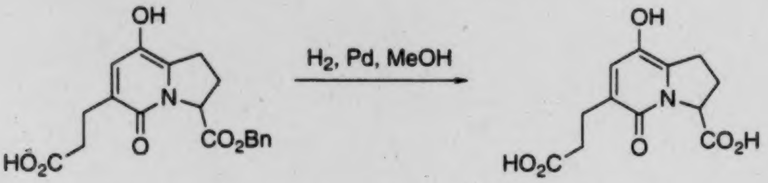
1,2,3,5-Tetrahydro-8-hydroxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoic acid (37.4) from 37.2b.



Freshly distilled CH_2Cl_2 (8 mL) was added to **37.2b** (0.0397 g, 0.112 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 5 min, TLC control, silica gel, 80:20 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.0583 g, 0.22 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow residue; the ketonic product (**37.3b**) could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

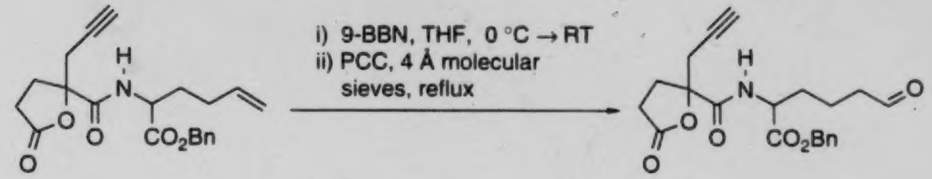
Dry Et_3N (1.0 mL, 7.17 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (10 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (1 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **37.4** (0.0379 g, 95%) as pure (^{1}H NMR), white crystals (mp 120-122 °C), spectroscopically identical to material obtained from **37.2a**.

3-Carboxy-1,2,3,5-tetrahydro-8-hydroxy-5-oxo-6-indolizinepropanoic acid (4.1).



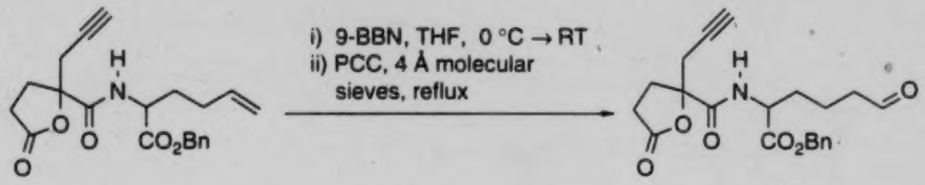
10% Pd-C (*ca.* 25 mg) was added to a stirred solution of **37.4** (0.1273 g, 0.36 mmol) in MeOH (40 mL). The reaction flask was flushed with hydrogen, and the mixture was stirred under hydrogen (balloon) until all of the starting material had been consumed (*ca.* 15 min, TLC control, silica gel, 95:5 EtOAc-AcOH). The mixture was filtered through a sintered glass frit (grade D). Evaporation of the filtrate gave a residue that was partitioned between 1% AcOH (15 mL) and $CHCl_3$ (5 mL). The aqueous layer was washed with $CHCl_3$ (2 x 5 mL), and evaporated to give a yellow solid that was recrystallized from water to afford **4.1** (0.0914 g, 94%) as pure (^{1}H NMR), light-yellow crystals: mp 163-165 °C; FTIR (microscope) 3600-2200, 1698, 1659 cm^{-1}; ^{1}H NMR (D_2O-CD_3CN, 400 MHz) δ 2.23-2.32 (m, 1 H), 2.44-2.72 (m, 5 H), 2.93-3.10 (twelve-line m, 2 H), 5.01 (dd, *J* = 9.8, 3.1 Hz, 1 H), 7.21 (s, 1 H); ^{13}C NMR (D_2O-CD_3CN, 75.5 MHz) δ 26.04 (t'), 27.09 (t'), 27.96 (t'), 33.39 (t'), 63.77 (d'), 128.9 (s'), 134.73 (d'), 134.94 (s'), 136.17 (s'), 160.08 (s'), 174.09 (s'), 177.57 (s'); exact mass *m/z* calcd for $C_{12}H_{13}NO_6$ 267.0743, found 267.0743.

6-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-DL-norleucine phenylmethyl ester (38.1a).



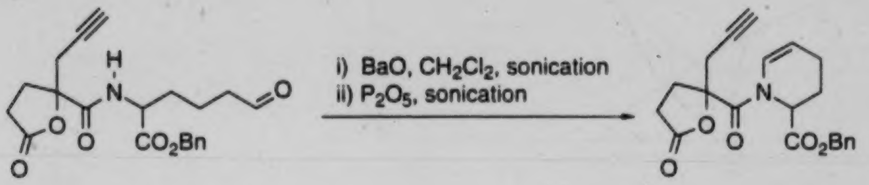
9-BBN (0.5 M in THF, 1.92 mL, 0.96 mmol) was added by syringe pump over *ca.* 4 min to a stirred and cooled (ice-water bath) solution of **32.1a** (0.3224 g, 0.87 mmol) in dry THF (10 mL) (Ar atmosphere). The cooling bath was removed and stirring was continued for 1 h. The mixture was then transferred by cannula to a stirred and cooled (ice-water bath) suspension of PCC (1.6977 g, 7.88 mmol) and crushed 4 Å molecular sieves (*ca.* 500 mg) in dry CH_2Cl_2 (10 mL). Additional dry CH_2Cl_2 (2 x 1 mL) was used as a rinse. The resulting mixture was transferred to an oil bath and stirred at reflux temperature for 1.5 h, allowed to cool to room temperature, and filtered through a pad (2.5 x 5 cm) of silica gel, using EtOAc as a rinse. Evaporation of the solvent, and flash chromatography of the residue over silica gel (2 x 25 cm), using 80:20 EtOAc-hexanes, gave **38.1a** (0.2657 g, 79%) as a pure (^{1}H NMR), colorless oil: FTIR ($CDCl_3$ cast) 3350, 3290, 2830, 2729, 1789, 1739, 1677 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48-1.66 (m, 2 H), 1.67-1.79 (m, 1 H), 1.80-1.95 (m, 1 H), 2.04 (t, J = 2.0 Hz, 1 H), 2.36-2.64 (m, 5 H), 2.66-2.84 [m, 3 H, including an apparent dd at δ 2.75 (A of an ABX system, J = 17.3, 2.7 Hz), and an apparent dd at δ 2.79 (B of an ABX system, J = 17.3, 2.7 Hz)], 4.54 (dt, J = 8.0, 5.1 Hz, 1 H), 5.12 and 5.18 (AB q, $\Delta\nu_{AB}$ = 22.4 Hz, J = 12.1 Hz, 2 H), 7.08 (d, J = 8.0 Hz, 1 H), 7.27-7.40 (m, 5 H), 9.68 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 17.66 (t'), 28.34 (t', two signals overlap), 29.54 (t'), 31.07 (t'), 42.79 (t'), 52.07 (d'), 67.35 (t'), 72.41 (d'), 77.06 (s'), 85.28 (s'), 128.42 (d'), 128.60 (d', two signals overlap), 135.03 (s'), 170.83 (s'), 170.99 (s'), 175.21 (s'), 201.32 (d'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_6$ (M + Na) 408.1423, found 408.1422.

6-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-DL-norleucine phenylmethyl ester (38.1b).



9-BBN (0.5 M in THF, 3.26 mL, 1.63 mmol) was added by syringe pump over *ca.* 4 min to a stirred and cooled (ice-water bath) solution of **32.1b** (0.5470 g, 1.48 mmol) in dry THF (10 mL) (Ar atmosphere). The cooling bath was removed and stirring was continued for 1 h. The mixture was then transferred by cannula to a stirred and cooled (ice-water bath) suspension of PCC (2.8700 g, 13.31 mmol) and crushed 4 Å molecular sieves (*ca.* 500 mg) in dry CH_2Cl_2 (10 mL). Additional dry CH_2Cl_2 (2 x 1 mL) was used as a rinse. The resulting mixture was transferred to an oil bath and stirred at reflux temperature for 1.5 h, allowed to cool to room temperature, and filtered through a pad (2.5 x 4 cm) of silica gel, using EtOAc as a rinse. Evaporation of the solvent, and flash chromatography of the residue over silica gel (2.5 x 25 cm), using 70:30 EtOAc-hexanes, gave **38.1b** (0.4679 g, 82%) as a pure (^{1}H NMR), colorless oil: FTIR ($CDCl_3$ cast) 3355, 3289, 2830, 2731, 1791, 1740, 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60-1.79 (m, 3 H), 1.89-1.99 (m, 1 H), 2.10 (t, J = 2.6 Hz, 1 H), 2.29-2.53 (m, 5 H), 2.58-2.72 (m, 1 H), 2.81 (A of an ABX system, apparent dd, J = 17.2, 2.6 Hz, 1 H), 2.85 (B of an ABX system, apparent dd, J = 17.2, 2.6 Hz, 1 H), 4.59 (dt, J = 8.2, 4.9 Hz, 1 H), 5.10 and 5.19 (AB q, $\Delta\nu_{AB}$ = 33.9 Hz, J = 12.1 Hz, 2 H), 7.00 (d, J = 8.2 Hz, 1 H), 7.27-7.41 (m, 5 H), 9.70 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 17.89 (t'), 28.13 (t'), 28.56 (t'), 30.03 (t'), 30.96 (t'), 42.90 (t'), 52.11 (d'), 67.47 (t'), 72.38 (d'), 85.46 (s'), 128.48 (d'), 128.66 (d'), 128.71 (d'), 135.07 (s'), 170.82 (s'), 170.92 (s'), 174.99 (s'), 201.21 (d'), the internal acetylene carbon signal overlaps the solvent signals; exact mass *m/z* calcd for $C_{21}H_{23}NO_6$ 385.1525, found 385.1526.

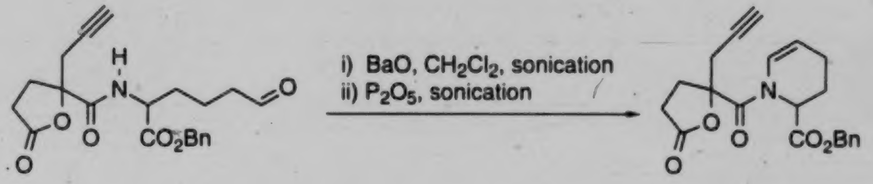
Phenylmethyl 1,2,3,4-Tetrahydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-2-pyridinecarboxylate (38.2a).



BaO (0.5231 g, 3.41 mmol) was tipped into a solution of **38.1a** (0.2657 g, 0.69 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.4922 g, 3.47 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 1.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (2 x 20 cm), using 40:60 EtOAc-hexanes, gave **38.2a** (0.2128 g, 84%) as a pure (^{1}H NMR), colorless oil, which was a mixture of rotamers: FTIR ($CHCl_3$ cast) 3281, 1793, 1743, 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80-2.16 [m, 4 H, including a t at δ 2.10 (*J* = 2.4 Hz), and a t at δ 2.14 (*J* = 2.3 Hz)], 2.24-2.79 (m, 5 H), 2.88-3.10 (m, 2 H), 4.99-5.53 [m, 4 H, including a dd at δ 5.02 (*J* = 8.1, 5.5 Hz, 1 H), and a br s at δ 5.52)], 7.12-7.22 [m, 1 H, including a d at δ 7.15 (*J* = 8.6 Hz), and a d at δ 7.20 (*J* = 8.6 Hz)], 7.28-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 18.78 (t'), 23.25 (t'), 23.95 (t'), 27.43 (t'), 27.92 (t'), 28.42 (t'), 30.27 (t'), 32.79 (t'), 53.79 (d'), 56.16 (d'), 67.26 (t'), 67.69

(t'), 72.40 (d'), 72.88 (d'), 86.41 (s'), 87.25 (s'), 108.55 (d'), 110.69 (d'), 124.35 (d'), 124.96 (d'), 128.25 (d'), 128.51 (d'), 128.62 (d'), 135.41 (s'), 167.65 (s'), 169.91 (s'), 175.26 (s'), the internal acetylene signals overlap the solvent signals; not all of the signals from the minor rotamer were observed; exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{21}NNaO_5$ (M + Na) 390.1317, found 390.1311.

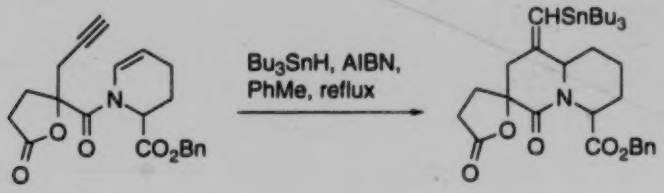
Phenylmethyl 1,2,3,4-Tetrahydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-2-pyridinecarboxylate (38.2b).



BaO (0.1432 g, 0.93 mmol) was tipped into a solution of **38.1b** (0.0690 g, 0.18 mmol) in dry CH_2Cl_2 (5 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.1396 g, 0.98 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 1 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (1 x 20 cm), using 50:50 EtOAc-hexanes, gave **38.2b** (0.0577 g, 89%) as a pure (^{1}H NMR), colorless oil: FTIR ($CHCl_3$ cast) 3282, 1793, 1741, 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86-2.14 [m, 4 H, including a t at δ 2.12 (J = 2.7 Hz)], 2.30-2.51 (m, 3 H), 2.63 (ddd, J = 18.0, 9.4, 5.5 Hz, 1 H), 2.88 (A of an ABX system, apparent dd, J = 17.1, 2.6 Hz, 1 H), 2.94 (B of an ABX system, apparent dd, J = 17.1, 2.6 Hz, 1 H), 3.01 (ddd, J = 13.2, 9.6, 5.5 Hz, 1 H), 5.03 (ddd, J = 8.4, 3.5, 2.1 Hz, 1 H), 5.09 and

5.20 (AB q, $\Delta\nu_{AB}$ = 43.3 Hz, *J* = 12.3 Hz, 2H), 5.27 (dd, *J* = 3.4, 3.4 Hz, 1 H), 7.20 (d, *J* = 8.6 Hz, 1 H), 7.27-7.40 (m, 5 H); ^{13}C NMR (CDCl$_3$, 75.5 MHz) δ 18.97 (t'), 23.53 (t'), 27.88 (t'), 28.93 (t'), 30.60 (t'), 53.65 (d'), 67.21 (t'), 72.89 (d'), 86.46 (s'), 109.21 (d'), 124.66 (d'), 128.06 (d'), 128.41 (d'), 128.66 (d'), 135.45 (s'), 166.98 (s'), 169.95 (s'), 174.99 (s'), the internal acetylene carbon signal overlaps the solvent signals; exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{21}NNaO_5$ (M + Na) 390.1317, found 390.1317.

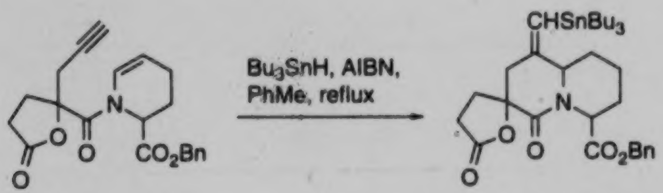
Phenylmethyl Octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.1a).



A solution of AIBN (0.0035 g, 0.02 mmol, 7.61 mM) and Bu$_3$SnH (0.08 mL, 0.30 mmol, 0.11 M) in dry PhMe (2.8 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **38.2a** (0.0519 g, 0.14 mmol) in PhMe (2.8 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 30:70 EtOAc-hexanes, gave racemic phenylmethyl octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (0.0691 g, 71%) as a colorless oil, containing slight impurities (^{1}H NMR): FTIR (CHCl$_3$

cast) 1789, 1744, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.84-1.04 (m, 15 H), 1.19-2.13 (m, 19 H), 2.41-2.60 (m, 2 H), 2.70-2.93 [m, 3 H, including an AB q at δ 2.75 and 2.79 ($\Delta\nu_{AB}$ = 13.2 Hz, *J* = 14.0 Hz), and a dt at δ 2.86 (*J* = 17.4, 9.6 Hz)], 4.11 (d, *J* = 9.6 Hz, 1 H), 4.22 (dd, *J* = 6.9, 4.6 Hz, 1 H), 5.09 and 5.21 (AB q, $\Delta\nu_{AB}$ = 41.7 Hz, *J* = 12.2 Hz, 2 H), 5.91 (vinyl proton, s, 1 H, Sn satellite signals at δ 5.84 and 5.98), 7.27-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 10.48 (t'), 13.67 (q'), 19.30 (t'), 24.52 (t'), 27.24 (t'), 28.76 (t'), 29.09 (t'), 29.99 (t'), 31.70 (t'), 42.97 (t'), 57.03 (d'), 60.80 (d'), 66.96 (t'), 82.09 (s'), 127.90 (d'), 128.28 (d'), 128.37 (d'), 128.53 (d'), 135.71 (s'), 145.87 (s'), 169.13 (s'), 170.75 (s'), 176.00 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{33}H_{49}NNaO_5{}^{120}Sn$ (M + Na) 682.2530, found 682.2533.

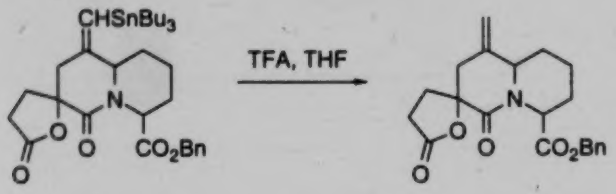
Phenylmethyl Octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.1b).



A solution of AIBN (0.0072 g, 0.04 mmol, 7.56 mM) and Bu_3SnH (0.16 mL, 0.59 mmol, 0.10 M) in dry PhMe (5.8 mL) was injected by syringe over *ca.* 15 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **38.2b** (0.1068 g, 0.29 mmol) in PhMe (5.8 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the solid, white residue over silica gel (2 x 20

cm), using 40:60 EtOAc-hexanes, gave racemic phenylmethyl octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (0.1474 g, 76%) as a white, amorphous solid, containing slight impurities (^{1}H NMR): FTIR (CH_2Cl_2 cast) 1786, 1739, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85-1.07 [m, 15 H, including a t at δ 0.90 (J = 7.3 Hz)], 1.31 (sextet, J = 7.3 Hz, 6 H), 1.37-1.67 (m, 9 H), 1.76-1.84 (m, 1 H), 1.87-1.98 (m, 2 H), 2.33 (d, J = 13.5 Hz, 1 H), 2.44-2.58 (m, 2 H), 2.62-2.71 (m, 1 H), 2.80-2.99 [m, 2 H, including a dt at δ 2.93 (J = 17.8, 9.9 Hz)], 4.09 (d, J = 11.3 Hz, 1 H), 5.15 and 5.23 (AB q, $\Delta\nu_{AB}$ = 34.0 Hz, J = 12.4 Hz, 2 H), 5.45-5.51 (m, 1 H), 5.87 (vinyl proton, d, J = 1.0 Hz, 1 H, Sn satellite signals at δ 5.80 and 5.94), 7.28-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 10.55 (t'), 13.68 (q'), 21.09 (t'), 26.13 (t'), 27.27 (t'), 28.80 (t'), 29.11 (t'), 32.01 (t'), 32.37 (t'), 42.97 (t'), 52.60 (d'), 60.84 (d'), 67.03 (t'), 82.04 (s'), 127.86 (d'), 128.00 (d'), 128.31 (d'), 128.65 (d'), 135.54 (s'), 145.70 (s'), 169.06 (s'), 170.24 (s'), 175.92 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{33}H_{49}NNaO_5{}^{120}Sn$ (M + Na) 682.2530, found 682.2538.

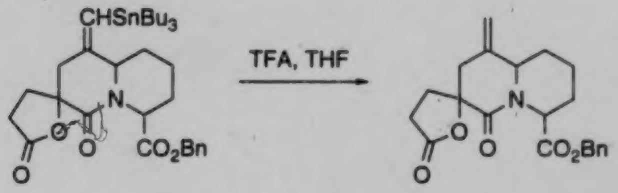
Phenylmethyl Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.2a).



Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the stannane **39.1a** (0.0512 g, 0.078 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 1 h no more stannane could be detected (TLC control, silica gel, 30:70 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using

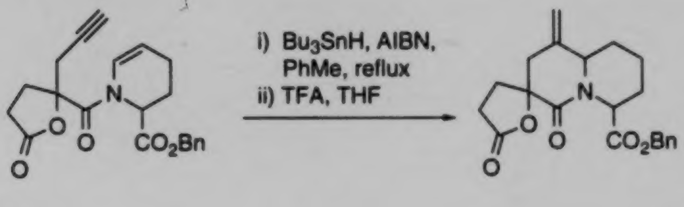
50:50 EtOAc-hexanes, gave **39.2a** (0.0270 g, 94%) as a pure (^{1}H NMR), colorless oil, spectroscopically identical to material obtained without isolation of the vinyl stannane.

Phenylmethyl Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.2b).



Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the vinyl stannane **39.1b** (0.1301 g, 0.20 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 60:40 EtOAc-hexanes, gave **39.2b** (0.0723 g, 100%) as a pure (^{1}H NMR), colorless oil, spectroscopically identical to material obtained without isolation of the vinyl stannane.

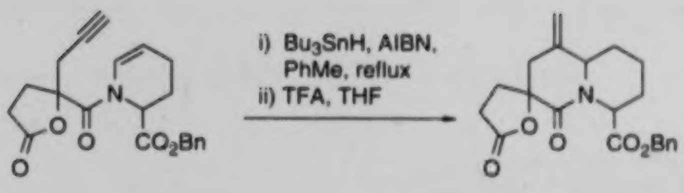
Phenylmethyl Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.2a) without isolation of the intermediate stanne.



A solution of AIBN (0.0043 g, 0.03 mmol, 7.86 mM) and Bu_3SnH (0.09 mL, 0.33 mmol, 0.10 M) in dry PhMe (3.33 mL) was injected by syringe over *ca.* 30 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **38.2a** (0.0611 g, 0.17 mmol) in PhMe (3.33 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (5 mL) (Ar atmosphere). After *ca.* 45 min no more vinyl stannane could be detected (TLC control, silica gel, 30:70 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 50:50 EtOAc-hexanes, gave **39.2a** (0.0442 g, 71%) as a pure (^{1}H NMR), colorless oil: FTIR ($CDCl_3$ cast) 1785, 1743, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60-1.82 (m, 3 H), 1.85-2.14 (m, 4 H), 2.40-2.51 (m, 2 H), 2.70 (d, J = 13.4 Hz, 1 H), 2.81 (dt, J = 17.8, 9.9 Hz, 1 H), 2.94 (d, J = 13.4 Hz, 1 H), 4.09 (d, J = 11.9 Hz, 1 H), 4.37 (dd, J = 6.1, 5.0 Hz, 1 H), 5.05-5.25 [m, 4 H, including a s at δ 5.09, a d at δ 5.11 (J = 1.3 Hz), and an AB q at 5.09 and 5.23 ($\Delta\nu_{AB}$ = 55.1 Hz, J = 12.2 Hz)], 7.28-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 18.19 (t'), 24.14 (t'), 27.62 (t'), 28.49 (t'), 32.38 (t'), 41.70 (t'), 56.28 (d'), 57.12 (d'), 66.98 (t'), 81.83 (s'), 113.86 (t'), 128.34 (d', two signals overlap), 128.57 (d'), 135.66 (s'), 138.50 (s'), 169.49 (s'), 170.92 (s'), 176.43 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_5$ (M + Na) 392.1474, found 392.1485.

Phenylmethyl Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (39.2b) without isolation of the intermediate stanne.

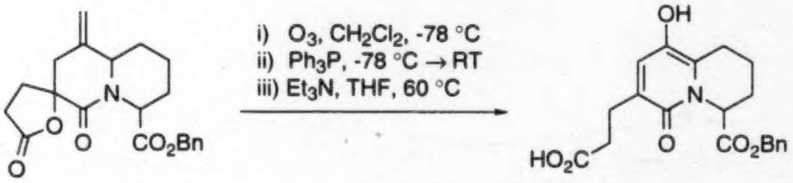


A solution of AIBN (0.0032 g, 0.02 mmol, 8.10 mM) and Bu_3SnH (0.07 mL, 0.26 mmol, 0.11 M) in dry PhMe (2.4 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **38.2b** (0.0449 g, 0.12 mmol) in PhMe (2.4 mL) (Ar atmosphere). Stirring at reflux was continued for 2.25 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (5 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 50:50 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 60:40 EtOAc-hexanes, gave **39.2b** (0.0410 g, 92%) as a pure (^{1}H NMR), colorless oil: FTIR ($CDCl_3$ cast) 1786, 1737, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32-1.49 (m, 2 H), 1.56-1.68 (m, 1 H), 1.73-1.86 (m, 1 H), 1.91-2.06 (m, 2 H), 2.30-2.41 (m, 2 H), 2.52 (ddd, J = 17.8, 9.9, 2.8 Hz, 1 H), 2.62 (d, J = 12.9 Hz, 1 H), 2.86 (dt, J = 17.8, 10.2 Hz, 1 H), 2.94 (d, J = 12.9 Hz, 1 H), 4.09 (d, J = 9.7 Hz, 1 H), 5.07 (d, J = 1.2 Hz, 1 H), 5.09 (s, 1 H), 5.16 and 5.20 (AB q, $\Delta\nu_{AB}$ = 17.0 Hz, J = 12.4 Hz, 2 H), 5.46 (dd, J = 5.8, 1.6 Hz, 1 H), 7.27-7.41 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.57 (t'), 26.13 (t'), 28.44 (t'), 30.32 (t'), 32.93 (t'), 41.46 (t'), 52.50 (d'), 57.81 (d'), 67.15 (t'), 81.91 (s'), 113.91 (t'), 128.04 (d'), 128.38 (d'), 128.66 (d'), 135.40 (s'), 138.31 (s'), 169.71 (s'), 170.21 (s'), 176.39 (s'); exact mass (HR electrospray) *m/z* calcd for

$C_{21}H_{23}NNaO_5$ 392.1474, found 392.1469.

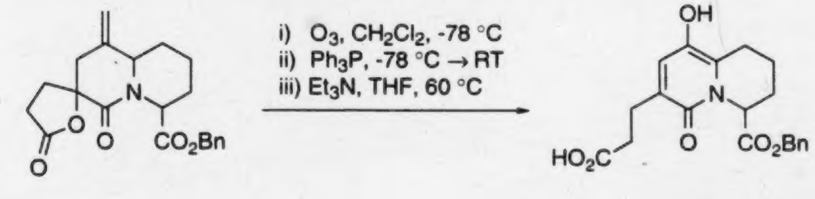
1,3,4,6-Tetrahydro-9-hydroxy-4-[(phenylmethoxy)carbonyl]-6-oxo-2*H*-quinolizine-7-propanoic acid (39.4) from 39.2a.



Freshly distilled CH_2Cl_2 (10 mL) was added to **39.2a** (0.0533 g, 0.14 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 3 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.0763 g, 0.29 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

Dry Et_3N (1.0 mL, 7.17 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (10 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (1.5 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **39.4** (0.0493 g, 93%) as a pure (^{1}H NMR), colorless oil, spectroscopically identical to material obtained from **39.2b**.

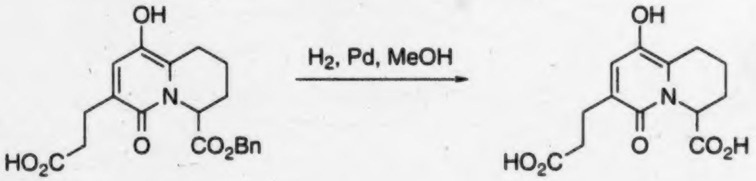
1,3,4,6-Tetrahydro-9-hydroxy-4-[(phenylmethoxy)carbonyl]-6-oxo-2*H*-quinolizine-7-propanoic acid (39.4) from 39.2b.



Freshly distilled CH_2Cl_2 (10 mL) was added to **39.2b** (0.1052 g, 0.28 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 3 min, TLC control, silica gel, 60:40 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.1495 g, 0.57 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

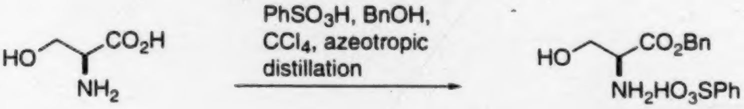
Dry Et_3N (2.0 mL, 14.35 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (20 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (1.5 x 15 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **39.4** (0.1015 g, 96%) as a pure (^{1}H NMR), light-yellow foam: FTIR ($CHCl_3$ cast) 3450-2300, 1737, 1705 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.46-1.62 (m, 1 H), 1.69-1.82 (m, 1 H), 2.01-2.14 (m, 1 H), 2.22-2.33 (m, 1 H), 2.50-2.58 (m, 2 H), 2.62-2.82 (m, 3 H), 2.88 (dt, J = 17.9, 4.5 Hz, 1 H), 5.11-5.24 [m, 3 H, including an AB q at δ 5.14 and 5.21 ($\Delta\nu_{AB}$ = 29.2 Hz, J = 12.3 Hz)], 7.24 (s, 1 H), 7.26-7.35 (m, 5 H); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 16.74 (t'), 23.81 (t'), 26.60 (t'), 27.34 (t'), 33.63 (t'), 57.13 (d'), 68.18 (t'), 127.79 (s'), 129.20 (d'), 129.33 (d'), 129.55 (d'), 130.73 (s'), 133.00 (d'), 137.14 (s'), 138.26 (s'), 161.89 (s'), 172.38 (s', two signals overlap); exact mass (HR electrospray) *m/z* calcd for $C_{20}H_{21}NNaO_6$ (M + Na) 394.1267, found 394.1269.

4-Carboxy-1,3,4,6-tetrahydro-9-hydroxy-6-oxo-2*H*-quinolizine-7-propanoic acid (4.2).



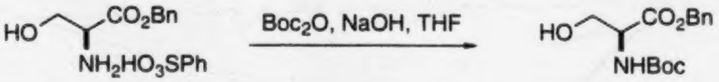
10% Pd-C (*ca.* 25 mg) was added to a stirred solution of **39.4** (0.0356 g, 0.096 mmol) in MeOH (6 mL). The reaction flask was flushed with hydrogen, and the mixture was stirred under hydrogen (balloon) until all of the starting material had been consumed (*ca.* 30 min, TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). The mixture was filtered through a sintered glass frit (grade D) and evaporated. Flash chromatography of the residue over reverse phase C-18 silica gel (Toronto Research Chemicals Inc., 10% capped with TMS) (1 x 20 cm), using 90:10 water-MeCN, gave **4.2** (0.0259 g, 96%) as a pure (^{1}H NMR), colorless oil: FTIR ($CHCl_3$-MeOH cast) 3650-2400, 1717 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 1.52-1.66 (m, 1 H), 1.72-1.85 (m, 1 H), 2.02-2.15 (m, 1 H), 2.21-2.33 (m, 1 H), 2.54-2.77 (m, 5 H), 2.86 (dt, J = 18.3, 5.0 Hz, 1 H), 5.05 (dd, J = 6.4, 3.8 Hz, 1 H), 7.30 (s, 1 H); ^{13}C NMR (D_2O, 100.6 MHz) δ 15.70 (t'), 23.30 (t'), 25.66 (t'), 26.03 (t'), 32.95 (t'), 57.15 (d'), 126.40 (s'), 132.21 (s'), 132.99 (d'), 137.28 (s'), 161.37 (s'), 175.70 (s'), 178.12 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{13}H_{16}NNaO_6$ (M + H) 282.0978, found 282.0986.

L-Serine Benzyl Ester Benzenesulfonate (41.2).



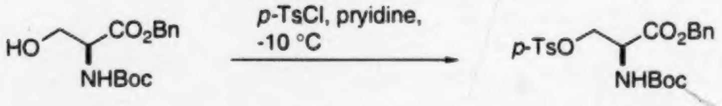
$PhSO_3H$ (42.3100 g, 267.48 mmol), L-serine (21.3102 g, 202.78 mmol), BnOH (100 mL, 966.34 mmol), and dry CCl_4 (125 mL) were added to a two-necked round-bottomed flask containing a magnetic stirring bar and fitted with an addition funnel containing dry CCl_4 and a simple distillation head and condenser. The mixture was distilled with continuous addition of fresh CCl_4 (the total volume added was *ca.* 350 mL), at a rate equal to the distillation rate, until no more water was generated (*ca.* 4 h). Completion of water formation was marked by an increase in the bp from 64 °C (the bp of the CCl_4-water azeotrope is *ca.* 66 °C) to 74 °C (the bp of CCl_4 is *ca.* 76 °C). The mixture was cooled to room temperature and the solvent was evaporated (<0.1 mm Hg). Et_2O (200 mL) was added to the residual viscous liquid. The resulting mixture was shaken vigorously, and then stored at -5 °C for 12 h, by which time a white precipitate had formed. The precipitate was collected, air dried, recrystallized from *i*-PrOH-Et_2O, and dried (<0.1 mm Hg) to give L-serine benzyl ester benzenesulfonate (55.9056 g, 78%) as pure (^1H NMR DMC V-53-B), fine white crystals: mp 95-97 °C; $[\alpha]^{25}_D = -2.63°$ (*c* 6.28, EtOH); FTIR ($CHCl_3$ cast) 3650-2500, 1750 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.85 (A of an ABX system, apparent dd, *J* = 12.5, 4.6 Hz, 1 H), 3.97 (B of an ABX system, apparent dd, *J* = 12.5, 3.0 Hz, 1 H), 4.13 (br s, 1 H), 4.80-5.20 (br s, 1 H), 4.98 and 5.03 (AB q, $\Delta\nu_{AB}$ = 18.1 Hz, *J* = 12.3 Hz, 2 H), 7.14-7.31 (m, 8 H), 7.77-7.82 (m, 2 H), 8.07 (br s, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 55.52 (d'), 59.81 (t'), 68.17 (t'), 126.02 (d'), 128.21 (d'), 128.44 (d'), 128.52 (d'), 130.55 (d'), 134.59 (s'), 143.56 (s'), 167.84 (s'), two d' in the aromatic region overlap; exact mass *m/z* calcd for $C_{10}H_{14}NO_3$ (M - $C_6H_5O_3S$) 196.0974, found 196.0972.

N-[(1,1-Dimethylethoxy)carbonyl]-L-serine phenylmethyl ester (41.3).



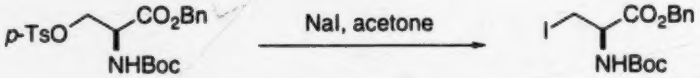
Aqueous NaOH (1 M, 125 mL) was added over *ca*. 5 min to a stirred and cooled (ice-water bath) solution of [(*t*-BuO)CO]$_2$O (32.8103 g, 150.33 mmol) and L-serine benzyl ester benzenesulfonate (47.3602 g, 133.99 mmol) in distilled THF (250 mL). After 15 min the cooling bath was removed and stirring was continued for 4 h. The solvent was evaporated, and the residue was dissolved in EtOAc (400 mL) and acidified with 10% (w/v) aqueous $KHSO_4$. The aqueous phase was extracted with EtOAc (2 x 200 mL), and the combined organic extracts were washed with water (2 x 300 mL) and saturated aqueous NaCl (300 mL), dried ($MgSO_4$), and evaporated, to give an off-white solid. This was recrystallized from EtOAc-hexanes to afford *N*-[(1,1-dimethylethoxy)carbonyl]-L-serine phenylmethyl ester (36.8012 g, 93%) as a pure (^{1}H NMR DMC V-85-B), fine, white powder: mp 74-76 °C; $[\alpha]^{25}_D = -15.04°$ (*c* 3.67, EtOH); FTIR ($CHCl_3$ cast) 3625-3150, 1775-1650 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H), 2.48 (br s, 1 H), 3.90 (A of an ABX system, apparent dd, *J* = 11.2, 3.4 Hz, 1 H), 3.98 (B of an ABX system, apparent dd, *J* = 11.2, 3.7 Hz, 1 H), 4.42 (br s, 1 H), 5.20 and 5.22 (AB q, $\Delta\nu_{AB}$ = 6.7 Hz, *J* = 12.4 Hz, 2 H), 5.52 (br d, *J* = 5.4 Hz, 1 H), 7.27-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.30 (q'), 55.93 (d'), 63.58 (t'), 67.40 (t'), 80.37 (s'), 128.20 (d'), 128.49 (d'), 128.65 (d'), 135.26 (s'), 155.76 (s'), 170.73 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{22}NO_5$ (M + H) 296.1498, found 296.1500.

N-[(1,1-Dimethylethoxy)carbonyl]-L-serine phenylmethyl ester 4-methylbenzenesulfonate ester (41.4).



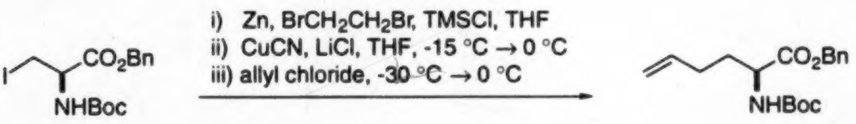
p-TsCl (39.7114 g, 208.30 mmol) was added to a stirred and cooled (-10 °C) solution of the above ester (41.0233 g, 138.91 mmol) in dry pyridine (290 mL) (Ar atmosphere). The resulting solution was stirred at -10 °C for 21 h, and then poured into ice-water (1000 mL). The mixture was stirred for *ca.* 3 h, and the resulting light-pink solid was collected, washed with distilled water, and air dried for 16 h. Recrystallization from EtOH gave *N*-[(1,1-dimethylethoxy)carbonyl]-L-serine phenylmethyl ester 4-methylbenzenesulfonate ester (53.6995, 86%) as pure (^{1}H NMR DMC V-95-A), white crystals: mp 94-96 °C; $[\alpha]^{25}_D = -1.21°$ (*c* 1.00, EtOH); FTIR ($CHCl_3$ cast) 3382, 1750, 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H), 2.44 (s, 3 H), 4.31 (A of an ABX system, apparent dd, J = 10.1, 3.0 Hz, 1 H), 4.42 (B of an ABX system, apparent dd, J = 10.1, 3.0 Hz, 1 H), 4.51-4.58 (m, 1 H), 5.10 and 5.18 (AB q, $\Delta\nu_{AB}$ = 31.8 Hz, J = 12.2 Hz, 2 H), 5.32 (d, J = 8.2 Hz, 1 H), 7.27-7.40 (m, 7 H), 7.72 (d, J = 7.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.68 (q'), 28.24 (q'), 53.15 (d'), 67.88 (t'), 69.52 (t'), 80.52 (s'), 128.01 (d'), 128.32 (d'), 128.61 (d'), 128.66 (d'), 129.96 (d'), 132.41 (s'), 134.84 (s'), 145.15 (s'), 154.98 (s'), 168.48 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{22}H_{27}NNaO_7S$ (M + Na) 472.1406, found 472.1417.

N-[(1,1-Dimethylethoxy)carbonyl]-3-iodo-L-alanine phenylmethyl ester (41.5).



NaI (4.2391 g, 28.28 mmol) in dry acetone (22 mL) was added dropwise by cannula to a stirred solution of the above ester (8.4563 g, 18.81 mmol) in dry acetone (22 mL) (Ar atmosphere). The resulting solution was stirred in the dark for 24 h, filtered through a sintered glass frit (grade C), and evaporated. The residue was partitioned between EtOAc (100 mL) and water (75 mL), and the organic phase was washed successively with water (75 mL), aqueous $Na_2S_2O_3$ solution (1 M, 75 mL), water (2 x 75 mL), and saturated aqueous NaCl solution (75 mL), dried ($MgSO_4$), and evaporated (< 0.1 mm Hg) to give a light-yellow oil that solidified after *ca.* 1 hr. The solid was recrystallized from EtOAc-Hexanes to give **41.5** (6.1747 g, 81%) as pure (^{1}H NMR DMC VII-49-A), white crystals: mp 78-80 °C; $[\alpha]^{25}_D = -17.63°$ (*c* 1.73, EtOH); FTIR ($CHCl_3$ cast) 3379, 1746, 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9 H), 3.56 (A of an ABX system, apparent dd, J = 10.3, 3.9 Hz, 1 H), 3.60 (B of an ABX system, apparent dd, J = 10.3, 3.6 Hz, 1 H), 4.55 (dt, J = 7.4, 3.8 Hz, 1 H), 5.20 and 5.23 (ABq, $\Delta\nu_{AB}$ = 13.5 Hz, J = 12.1 Hz, 2 H), 5.36 (d, J = 7.4 Hz, 1 H), 7.32-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 7.81 (t'), 28.32 (q'), 53.75 (d'), 68.01 (t'), 80.55 (s'), 128.62 (d'), 128.70 (d'), 134.91 (s'), 154.89 (s'), 169.52 (s'), two d' in the aromatic region are presumed to be overlapping; exact mass *m/z* calcd for $C_{15}H_{20}INO_4$: 405.0437, found 405.0446.

Phenylmethyl (*S*)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-5-hexenoate (41.7).


I
CO2Bn
NHBoc
i) Zn, BrCH2CH2Br, TMSCl, THF
ii) CuCN, LiCl, THF, -15 °C → 0 °C
iii) allyl chloride, -30 °C → 0 °C
CO2Bn
NHBoc

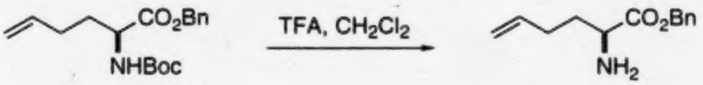
Zn dust (0.7452 g, 11.40 mmol) was added to a round-bottomed flask containing a magnetic stirring bar. The flask was sealed with a rubber septum, evacuated with an oil-pump, and refilled with Ar. The evacuation-filling process was repeated twice more, and then the system was placed under a static pressure of Ar. Dry THF (4 mL) and 1,2-

dibromoethane (0.05 mL, 0.58 mmol) were added. The resulting mixture was heated to boiling with the aid of a heat gun, and then allowed to cool for 1 min. The heating-cooling process was repeated three more times, and then the flask was allowed to cool for an additional 5 min. Freshly distilled, dry Me_3SiCl (0.07 mL, 0.55 mmol) was added, and the resulting mixture was stirred for 10 min and then placed in an oil bath set at 38 °C. Stirring was continued for 10 min. A solution of **41.5**[43] (1.1526 g, 2.84 mmol) in dry THF (5 mL) was added by cannula over *ca.* 1 min, and additional THF (3 x 1 mL) was used as a rinse. The resulting mixture was stirred at 38 °C (Ar atmosphere) until all the starting material had been consumed (*ca.* 6 h, TLC control, silica gel, 10:90 EtOAc-hexanes).

The above solution of alkylzinc iodide (**41.6**) was transferred by cannula over *ca.* 1 min to a stirred and precooled (bath temperature -15 °C) solution of CuCN (0.2811 g, 3.14 mmol) and LiCl (0.2661 g, 6.28 mmol) in dry THF (7 mL), and further portions of THF (2 x 1 mL) were used as a rinse to complete the transfer. The reaction flask was removed from the cold bath and placed in another at 0 °C. Stirring was continued for 15 min, and the reaction flask was then transferred to a cold bath at -30 °C. Stirring was continued for 10 min, allyl chloride (0.46 mL, 5.64 mmol) was added and, finally, the reaction flask was transferred to a cold bath at 0 °C. Stirring was continued for 14 h. The resulting solution was acidified with hydrochloric acid (2 M), and shaken with water (25 mL) and EtOAc (25 mL). The resulting white precipitate was filtered off, using a Whatman #2 filter paper, and the aqueous filtrate was extracted with EtOAc (2 x 15 mL), and filtered, as before, after each extraction to remove a small amount of precipitate. The combined organic extracts were washed with water (30 mL), filtered (as above), washed with saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 30 cm), using 15:85 EtOAc-hexanes, gave **41.7** (0.7631 g, 84%) as a pure (^{1}H NMR DMC VII-61-A), colorless oil: $[\alpha]^{25}_D = -18.90°$ (*c* 3.10, EtOH); FTIR ($CHCl_3$ cast) 3366, 1741, 1716 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44

(s, 9 H), 1.67-1.78 (m, 1 H), 1.86-1.98 (m, 1 H), 2.00-2.17 (m, 2 H), 4.32-4.42 (m, 1 H), 4.95-5.05 (m, 2 H), 5.09 (d, J = 7.7 Hz, 1 H), 5.14 and 5.20 (AB q, $\Delta\nu_{AB}$ = 26.0 Hz, J = 12.4 Hz, 2 H), 5.76 (ddt, J = 17.1, 10.3, 6.6 Hz, 1 H), 7.29-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.31 (q'), 29.43 (t'), 31.93 (t'), 53.12 (d'), 66.98 (t'), 79.85 (s'), 115.68 (t'), 128.26 (d'), 128.40 (d'), 128.58 (d'), 135.43 (s'), 136.94 (d'), 155.30 (s'), 172.61 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{18}H_{25}NNaO_4$ (M + Na) 342.1681, found 342.1681.

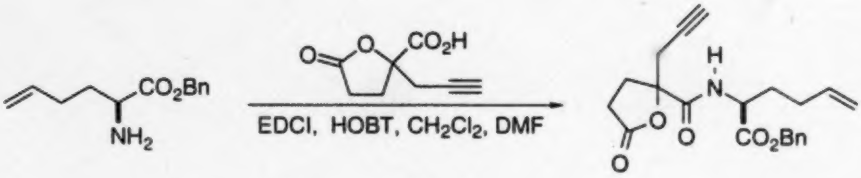
Phenylmethyl (*S*)-2-Amino-5-hexenoate (40.1).



CF_3CO_2H (5 mL) was added over *ca.* 5 min to a stirred and cooled (ice-water bath) solution of **41.7** (0.2871 g, 0.90 mmol) in freshly distilled CH_2Cl_2 (5 mL). The ice-water bath was removed and stirring was continued until all the starting material had been consumed (*ca.* 30 min, TLC control, silica gel, 15:85 EtOAc-hexanes), by which time the mixture had warmed to room temperature. The solution was evaporated, and the residue was dissolved in EtOAc (10 mL), washed with saturated aqueous $NaHCO_3$ (2 x 10 mL), water (2 x 10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and evaporated (<0.1 mm Hg). Flash chromatography of the residue over silica gel (1.5 x 20 cm), using 90:10 EtOAc-hexanes, gave **40.1** (0.1912 g, 97%) as a pure (^{1}H NMR DMC VIII-37-A), colorless oil: $[\alpha]^{25}_D = 7.94°$ (*c* 5.19, EtOH); FTIR ($CHCl_3$ cast) 3383, 3318, 1734, cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.58-1.72 [m, 3 H, including a s (NH_2) at δ 1.68], 1.78-1.89 (m, 1 H), 2.05-2.20 (m, 2 H), 3.48 (dd, J = 7.7, 5.3 Hz, 1 H), 4.93-5.06 [m, 2 H, including a dd at δ 5.01 (J = 17.2, 1.6 Hz)], 5.12 and 5.14 (AB q, $\Delta\nu_{AB}$ = 7.9 Hz, J = 12.3 Hz, 2 H), 5.76 (ddt, J = 17.2, 10.3, 6.6 Hz, 1 H), 7.26-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 29.77 (t'), 34.03 (t'), 53.84 (d'), 66.58 (t'), 115.33 (t'), 128.23

(d'), 128.31 (d'), 128.55 (d'), 135.72 (s'), 137.47 (d'), 175.95 (s'); exact mass *m/z* calcd for $C_{13}H_{17}NO_2$ 219.1259, found 219.1262.

Phenylmethyl (2*S*)-2-[[[Tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]amino]-5-hexenoate (40.2a, 40.2b).



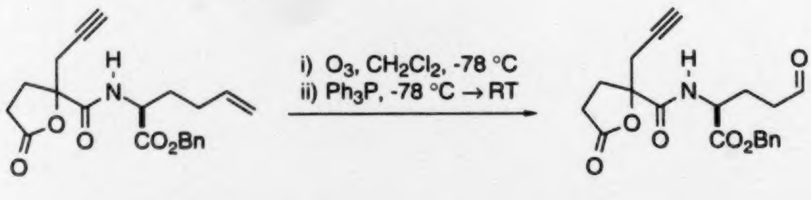
Lactone acid **32.2** (2.8235 g, 16.79 mmol), 1-hydroxybenzotriazole (2.2733 g, 16.82 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.2349 g, 16.87 mmol) were added in that order to a stirred solution of **40.1** (3.3419 g, 15.24 mmol) in a mixture of dry CH_2Cl_2 (112 mL) and freshly distilled, dry (stored over 4 Å molecular sieves) DMF (29 mL) (Ar atmosphere). Stirring at room temperature was continued for 12 h, the mixture was washed with water (2 x 60 mL) and saturated aqueous NaCl (60 mL), dried ($MgSO_4$), and evaporated, to give a dark purple-oil. Chromatography of the oil over silica gel (4.5 x 30 cm), repeated three times, using 3.5:3.5:4 CH_2Cl_2-Et_2O-hexanes, gave the faster-eluting diastereomer (**40.2a**) (2.7374 g, 49%) as a pure (^1H NMR DMC VIII-45-A), colorless oil, and the slower-eluting diastereomer (**40.2b**) (2.6112 g, 46%), also as a pure (^1H NMR DMC VIII-45-B), colorless oil.

Compound **40.2a** had: $[\alpha]^{25}_D = -21.01°$ (*c* 2.68, EtOH); FTIR ($CHCl_3$ cast) 3350, 3295, 1789, 1741, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.78-1.88 (m, 1 H), 1.92-2.10 (m, 4 H), 2.43-2.59 (m, 3 H), 2.69-2.84 [m, 3 H, including an apparent dd at δ 2.76 (A of an ABX system, *J* = 17.3, 2.6 Hz), and an apparent dd at δ 2.80 (B of an ABX system, *J* = 17.3, 2.6 Hz)], 4.57 (dt, *J* = 8.1, 5.0 Hz, 1 H), 4.96-5.03 (m, 2 H), 5.12 and

5.19 (AB q, $\Delta\nu_{AB}$ = 24.8 Hz, *J* = 12.2 Hz, 2 H), 5.72 (ddt, *J* = 17.8, 9.7, 6.4 Hz, 1 H), 6.98 (d, *J* = 8.1 Hz, 1 H), 7.28-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.38 (t', two signals overlap), 29.46 (t'), 29.59 (t'), 30.93 (t'), 52.05 (d'), 67.28 (t'), 72.43 (d'), 77.19 (s'), 85.31 (s'), 116.16 (t'), 128.37 (d'), 128.54 (d'), 128.61 (d'), 135.14 (s'), 136.50 (d'), 170.83 (s'), 171.02 (s'), 175.03 (s'); exact mass *m/z* calcd for $C_{21}H_{23}NO_5$ 369.1576, found 369.1565.

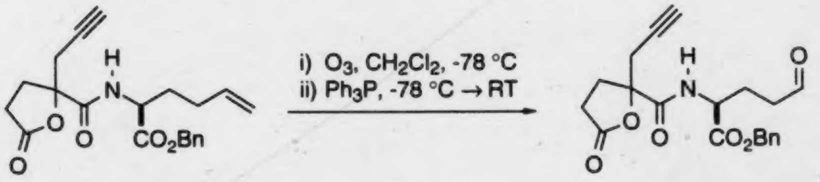
Compound **40.2b** had: $[\alpha]^{25}_D$ = 0.63° (*c* 3.98, EtOH); FTIR ($CHCl_3$ cast) 3350, 3297, 1790, 1740, 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.76-1.87 (m, 1 H), 1.95-2.14 [m, 4 H, including a t at δ 2.08 (*J* = 2.6 Hz)], 2.30-2.52 (m, 3 H), 2.58-2.71 (m, 1 H), 2.76-2.89 (m, 2 H), 4.61 (dt, *J* = 8.4, 4.7 Hz, 1 H), 4.97-5.05 (m, 2 H), 5.10 and 5.19 (AB q, $\Delta\nu_{AB}$ = 35.9 Hz, *J* = 12.2 Hz, 2 H), 5.74 (ddt, *J* = 16.6, 9.7, 6.6 Hz, 1 H), 6.93 (d, *J* = 8.4 Hz, 1 H), 7.27-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.09 (t'), 28.43 (t'), 29.52 (t'), 29.96 (t'), 30.77 (t'), 51.96 (d'), 67.28 (t'), 72.24 (d'), 77.40 (s'), 85.40 (s'), 116.27 (t'), 128.35 (d'), 128.54 (d'), 128.63 (d'), 135.13 (s'), 136.44 (d'), 170.66 (s'), 171.13 (s'), 175.05 (s'); exact mass *m/z* calcd for $C_{21}H_{23}NO_5$ 369.1576, found 369.1574.

5-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-L-norvaline phenylmethyl ester (42.1a).



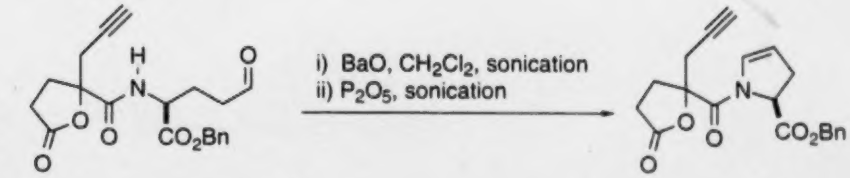
Freshly distilled CH_2Cl_2 (15 mL) was added to **40.2a** (0.5333 g, 1.44 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 8 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.7672 g, 2.93 mmol) was added. The cooling bath was removed and stirring was continued for 4 h, by which time the mixture had warmed to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (3 x 20 cm), using 90:10 EtOAc-hexanes, gave **42.1a** (0.5199 g, 97%) as an oil, containing trace amounts of what we take to be the corresponding *E* and *Z* enol tautomers (^{1}H NMR DMC VIII-73-A): $[\alpha]^{25}_D = -12.32°$ (*c* 3.79, $CHCl_3$); FTIR ($CHCl_3$ cast) 3356, 3288, 2834, 2731, 1789, 1740, 1677 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96-2.16 [m, 2 H, including a t at δ 2.03 (*J* = 2.7 Hz)], 2.24 (ddt, *J* = 13.9, 6.8, 4.9 Hz, 1 H), 2.39-2.62 (m, 5 H), 2.67-2.91 [m, 3 H, including an apparent dd at δ 2.74 (A of an ABX system, *J* = 17.2, 2.7 Hz), and an apparent dd at δ 2.78 (B of an ABX system, *J* = 17.2, 2.7 Hz)], 4.55 (dt, *J* = 8.0, 5.2 Hz, 1 H), 5.13 and 5.18 (AB q, $\Delta\nu_{AB}$ = 18.4 Hz, *J* = 12.1 Hz, 2 H), 7.20 (d, *J* = 8.0 Hz, 1 H), 7.28-7.40 (m, 5 H), 9.68 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 24.02 (t'), 28.29 (t'), 28.44 (t'), 29.43 (t'), 39.84 (t'), 51.91 (d'), 67.49 (t'), 72.42 (d'), 77.21 (s'), 85.23 (s'), 128.50 (d'), 128.56 (d'), 128.64 (d'), 134.99 (s'), 170.50 (s'), 171.09 (s'), 175.16 (s'), 200.49 (d'); exact mass *m/z* calcd for $C_{20}H_{21}NO_6$ 371.1369, found 371.1371.

5-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-L-norvaline phenylmethyl ester (42.1b).



Freshly distilled CH_2Cl_2 (10 mL) was added to **40.2b** (0.3622 g, 0.98 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 10 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.5160 g, 1.97 mmol) was added. The cooling bath was removed and stirring was continued for 2 h, by which time the mixture had warmed to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (3 x 25 cm), using 70:30 EtOAc-hexanes, gave **42.1b** (0.3495 g, 96%) as a pure oil, containing trace amounts of what we take to be the corresponding *E* and *Z* enol tautomers (^{1}H NMR): $[\alpha]^{25}_D = -4.57°$ (*c* 6.65, $CHCl_3$); FTIR ($CDCl_3$ cast) 3350, 3286, 2834, 2732, 1789, 1740, 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.95-2.12 [m, 2 H, including a t at δ 2.08 (J = 2.8 Hz)], 2.20-2.66 (m, 7 H), 2.80 (A of an ABX system, apparent dd, J = 16.9, 2.6 Hz, 1 H), 2.82 (B of an ABX system, apparent dd, J = 16.9, 2.6 Hz, 1 H), 4.58 (dt, J = 8.3, 4.8 Hz, 1 H), 5.10 and 5.16 (AB q, $\Delta\nu_{AB}$ = 23.7 Hz, J = 12.1 Hz, 2 H), 7.23 (d, J = 8.3 Hz, 1 H), 7.27-7.39 (m, 5 H), 9.68 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 23.84 (t'), 28.02 (t'), 28.33 (t'), 29.96 (t'), 39.80 (t'), 51.75 (d'), 67.45 (t'), 72.24 (d'), 77.50 (s'), 85.34 (s'), 128.37 (d'), 128.58 (d'), 128.63 (d'), 134.97 (s'), 170.58 (s'), 170.88 (s'), 175.05 (s'), 200.49 (d'); exact mass *m/z* calcd for $C_{20}H_{21}NO_6$ 371.1369, found 371.1372.

Phenylmethyl (2*S*)-2,3-Dihydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-1*H*-pyrrole-2-carboxylate (42.2a).

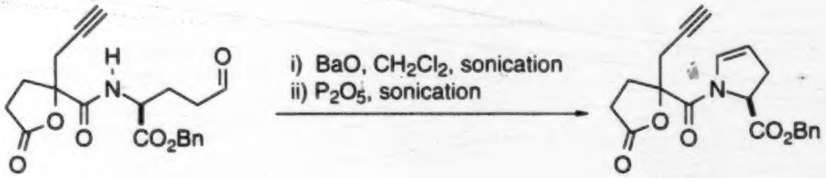


BaO (0.3412 g, 2.23 mmol) was tipped into a solution of **42.1a** (0.3865 g, 1.04 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.3922 g, 2.76 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde was being consumed (*ca.* 2.5 h, TLC control, silica gel, 60:40 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (2 x 25 cm), using 60:40 EtOAc-hexanes, gave **42.2a** (0.2612 g, 71%, 93% after correction for recovered starting material) as a pure (^{1}H NMR DMC VIII-79-A), colorless oil, which was a mixture of rotamers. The material should be used within 24 h. Further elution with 90:10 EtOAc-hexanes gave starting material (0.0878 g, 0.24 mmol) (^{1}H NMR DMC VIII-79-B) as a light-yellow oil.

Compound **42.2a** had: $[\alpha]^{25}_D = -83.09°$ (*c* 5.56, $CHCl_3$); FTIR ($CHCl_3$ cast) 3283, 1792, 1745, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.07-2.12 [m, 1 H, including a t at δ 2.10 (*J* = 2.6 Hz)], 2.36-2.78 [m, 5 H, including a dd at δ 2.64 (*J* = 17.4, 2.7 Hz), and a ddd at δ 2.72 (*J* = 17.4, 10.2, 6.4 Hz)], 2.79-3.24 (m, 3 H, including an apparent dd at δ 2.87 (A of an ABX system, *J* = 12.6, 2.6 Hz), and an apparent dd at δ 2.94 (B of an ABX system, *J* = 12.6, 2.6 Hz), and a ddt at δ 3.19 (*J* = 17.1, 11.2, 2.5 Hz)], 4.86-5.42 [m, 4 H, including a dd at δ 4.89 (*J* = 11.5, 4.2 Hz), an AB q at δ 5.07 and 5.20 ($\Delta\nu_{AB}$ = 52.7 Hz, *J* = 12.0 Hz), overlapping an AB q at δ 5.11 and 5.23 ($\Delta\nu_{AB}$ = 47.4 Hz, *J* = 12.2 Hz), overlapping a dd at δ 5.25 (*J* = 7.0, 2.7 Hz), a dd at δ 5.28 (J = 4.6, 2.6 Hz), and a dd at δ 5.39 (J = 11.1, 2.7 Hz)], 7.05-7.23 [m, 1 H, including a ddd at δ 7.07 (J = 4.4, 2.8, 1.6 Hz), and a dt at δ 7.21 (J = 4.4, 2.2 Hz)], 7.31-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.72 (t'), 28.07 (t'), 28.59 (t'), 29.34 (t'), 30.67 (t'), 31.21 (t'), 32.17 (t'), 36.39 (t'), 59.32 (d'), 59.71 (d'), 67.17 (t'), 67.86 (t'), 72.18 (d'), 72.79 (d'), 86.13 (s'), 86.28 (s'), 109.94 (d'), 111.33 (d'), 128.34 (d'), 128.50 (d'), 128.54 (d'), 128.63 (d'), 128.68 (d'), 129.44 (d'), 130.97 (d'), 135.16 (s'), 135.32 (s'), 165.94 (s'), 167.83 (s'), 170.22 (s'), 172.39 (s'), 175.14 (s'), 175.35 (s'), one pair of d' in the aromatic region overlap; the internal acetylene carbon signals overlap the solvent signals; exact mass *m/z* calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1261.

If the aldehyde solution is not pre-treated with BaO for *ca.* 1 h before addition of P_2O_5, the overall yield is <50%. In the absence of BaO, the P_2O_5 turns into a reddish-brown gummy material after sonication in CH_2Cl_2 for 1 h or more; when BaO is present, the P_2O_5 stays as a fine powdered suspension. If additional P_2O_5 is added to the system during the course of the reaction in an attempt to improve the conversion, an unidentified byproduct is formed, and the yield of **42.2a** is reduced.

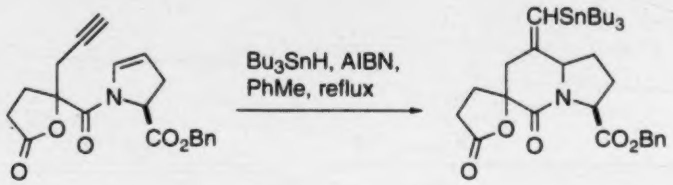
Phenylmethyl (2*S*)-2,3-Dihydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-1*H*-pyrrole-2-carboxylate (42.2b).



BaO (0.4101 g, 2.67 mmol) was tipped into a solution of **42.1b** (0.2321 g, 0.63 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80

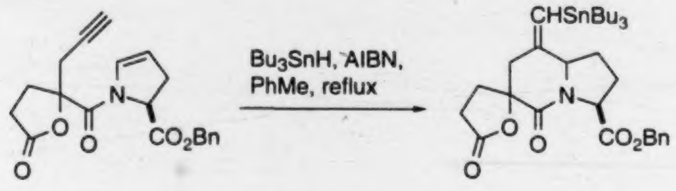
W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.3673 g, 2.59 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 2.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (1.5 x 20 cm), using 50:50 EtOAc-hexanes, gave **42.2b** (0.1944 g, 87%) as a pure (^{1}H NMR), colorless oil, which was a mixture of rotamers: $[\alpha]^{25}_D = -85.02°$ (*c* 2.69, $CHCl_3$); FTIR ($CDCl_3$ cast) 3283, 1793, 1747, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.04-2.13 [m, 1 H, including a t at δ 2.10 (J = 2.6 Hz)], 2.27-2.45 (m, 2 H), 2.49-2.62 (m, 2 H), 2.73-3.01 [m, 4 H, including an apparent dd at δ 2.78 (A of an ABX system, J = 17.1, 2.6 Hz), and an apparent dd at δ 2.88 (B of an ABX system, J = 17.1, 2.6 Hz), and a ddt at δ 2.96 (J = 18.4, 11.6, 2.2 Hz)], 4.96 (dd, J = 11.7, 5.2 Hz, 1 H), 5.06 and 5.26 (AB q, $\Delta\nu_{AB}$ = 77.7 Hz, J = 12.2 Hz, 2 H), 5.21 (dt, J = 4.5, 2.6 Hz, 1 H), 7.02-7.23 [m, 1 H, including a five line m at δ 7.03, and a dt at δ 7.20 (J = 4.5, 2.3 Hz)], 7.28-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.85 (t'), 28.66 (t'), 31.00 (t'), 31.77 (t'), 32.03 (t'), 32.87 (t'), 36.73 (t'), 59.55 (d'), 59.93 (d'), 67.15 (t'), 67.75 (t'), 72.21 (d'), 72.70 (d'), 76.95 (s'), 86.05 (s'), 86.35 (s'), 109.56 (d'), 111.10 (d'), 128.27 (d'), 128.40 (d'), 128.47 (d'), 128.65 (d'), 129.40 (d'), 131.14 (d'), 135.33 (s'), 166.47 (s'), 170.25 (s'), 175.03 (s'), not all signals for the minor rotamer were observed; exact mass *m/z* calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1269.

Phenylmethyl (3'*S*)-Octahydro-5,5'-dioxo-8'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylic acid (43.1a).



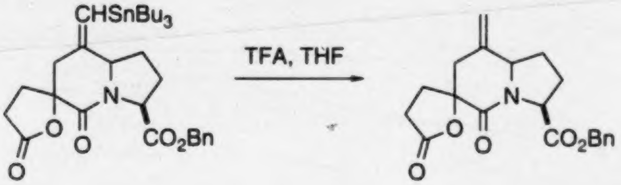
A solution of AIBN (0.0077 g, 0.05 mmol, 7.82 mM) and Bu_3SnH (0.17 mL, 0.63 mmol, 0.11 M) in dry PhMe (6.0 mL) was injected by syringe over *ca.* 30 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **42.2a** (0.1060 g, 0.30 mmol) in PhMe (6.0 mL) (Ar atmosphere). Stirring at reflux was continued for 2.75 h. A solution of AIBN (0.0079 g, 0.05 mmol, 16.0 mM) and Bu_3SnH (0.17 mL, 0.63 mmol, 0.21 M) in dry PhMe (3.0 mL) was then injected by syringe over *ca.* 30 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.75 h, TLC control, silica gel, 40:60 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the oily residue over silica gel (2.5 x 20 cm), using 30:70 EtOAc-hexanes, gave **43.1a** (0.1488 g, 77%) as a colorless oil, containing slight impurities (^{1}H NMR): $[\alpha]^{25}_D = 26.24°$ (*c* 2.63, $CHCl_3$); FTIR ($CHCl_3$ cast) 1789, 1744, 1664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87-0.97 [m, 15 H, including a t at δ 0.90 (*J* = 7.3 Hz)], 1.31 (sextet, *J* = 7.3 Hz, 6 H), 1.38-1.58 (m, 6 H), 1.78-2.01 (m, 2 H), 2.02-2.11 (m, 1 H), 2.16-2.30 (m, 2 H), 2.43-2.54 (m, 1 H), 2.64-2.96 (m, 4 H), 4.46 (dd, *J* = 11.0, 3.8 Hz, 1 H), 4.55 (d, *J* = 8.8 Hz, 1 H), 5.08 and 5.22 (AB q, $\Delta\nu_{AB}$ = 56.6 Hz, *J* = 12.3 Hz, 2 H), 5.84 (vinyl proton, d, *J* = 1.7 Hz, 1 H, Sn satellite signals at δ 5.76 and 5.89), 7.29-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 10.33 (t'), 13.70 (q'), 27.30 (t'), 28.26 (t'), 28.71 (t'), 29.13 (t'), 30.00 (t'), 30.49 (t'), 44.09 (t'), 58.39 (d'), 63.80 (d'), 67.09 (t'), 83.50 (s'), 125.73 (d'), 128.21 (d'), 128.44 (d'), 128.64 (d'), 135.50 (s'), 147.11 (s'), 166.94 (s'), 171.11 (s'), 176.29 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{32}H_{47}NNaO_5{}^{120}Sn$ (M + Na) 668.2374, found 668.2376.

Phenylmethyl (3'*S*)-Octahydro-5,5'-dioxo-8'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylic acid (43.1b).



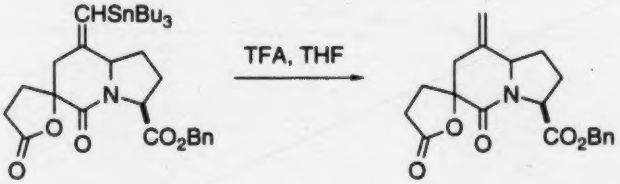
A solution of AIBN (0.0070 g, 0.04 mmol, 7.75 mM) and Bu_3SnH (0.15 mL, 0.56 mmol, 0.10 M) in dry PhMe (5.5 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **42.2b** (0.0967 g, 0.27 mmol) in PhMe (5.5 mL) (Ar atmosphere). Stirring at reflux was continued for 2.5 h. A solution of AIBN (0.0071 g, 0.04 mmol, 15.44 mM) and Bu_3SnH (0.15 mL, 0.56 mmol, 0.20 M) in dry PhMe (2.8 mL) was then injected by syringe over *ca.* 10 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the oily residue over silica gel (2 x 20 cm), using 35:65 EtOAc-hexanes, gave **43.1b** (0.1481 g, 85%) as a colorless oil, containing slight impurities (^1H NMR): $[\alpha]^{25}_D = -114.13°$ (*c* 2.81, $CHCl_3$); FTIR ($CHCl_3$ cast) 1787, 1747, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, *J* = 7.3 Hz, 9 H), 0.92-1.05 (m, 6 H), 1.36 (sextet, *J* = 7.3 Hz, 6 H), 1.37-1.60 (m, 6 H), 1.69-1.82 (m, 1 H), 1.85-1.97 (m, 2 H), 2.24-2.33 (five line m, 1 H), 2.35-2.45 (m, 1 H), 2.47-2.64 (m, 3 H), 2.82-2.94 (m, 1 H), 3.01-3.09 (m, 1 H), 4.47 (dd, *J* = 10.5, 4.8 Hz, 1 H), 4.56 (t, *J* = 8.5 Hz, 1 H), 5.14 and 5.21 (AB q, $\Delta\nu_{AB}$ = 30.0 Hz, *J* = 12.4 Hz, 2 H), 5.86 (vinyl proton, d, *J* = 1.4 Hz, 1 H, Sn satellite signals at δ 5.79 and 5.92), 7.28-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 10.42 (t'), 13.63 (q'), 27.22 (t'), 27.47 (t'), 28.55 (t'), 29.07 (t'), 31.03 (t'), 31.65 (t'), 44.17 (t'), 58.42 (d'), 63.91 (d'), 66.94 (t'), 82.75 (s'), 127.12 (d'), 128.08 (d'), 128.25 (d'), 128.56 (d'), 135.50 (s'), 146.06 (s'), 167.09 (s'), 171.13 (s'), 175.85 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{32}H_{47}NNaO_5{}^{120}Sn$ (M + Na) 668.2374, found 668.2372.

Phenylmethyl (3'*S*)-Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (43.2a).



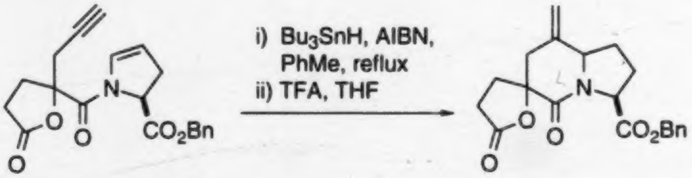
Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of **43.1a** (0.0633 g, 0.098 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 45 min no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1 x 20 cm), using 60:40 EtOAc-hexanes, gave **43.2a** (0.0339 g, 97%) as pure (^{1}H NMR), white crystals (mp 136-139 °C), spectroscopically identical to material obtained without isolation of the vinyl stannane.

Phenylmethyl (3'*S*)-Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (43.2b).



Dry CF_3CO_2H (1.5 mL) was injected rapidly into a stirred solution of **43.1b** (0.1212 g, 0.19 mmol) in THF (15 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 35:65 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 80:20 EtOAc-hexanes, gave **43.2b** (0.0662 g, 100%) as pure (^1H NMR), white crystals (mp 118-121 °C), spectroscopically identical to material obtained without isolation of the vinyl stannane.

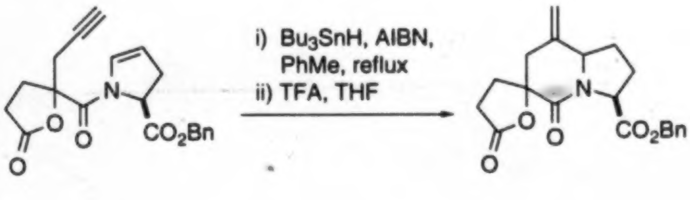
Phenylmethyl (3'*S*)-Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (43.2a) without isolation of the intermediate stannane.



A solution of AIBN (0.0281 g, 0.17 mmol, 7.79 mM) and Bu_3SnH (0.63 mL, 2.34 mmol, 0.11 M) in dry PhMe (22 mL) was injected by syringe over *ca.* 2 min into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **42.2a** (0.3911 g, 1.11 mmol) in PhMe (22 mL) (Ar atmosphere). Stirring at reflux was continued for 3 h. A solution of AIBN (0.0277 g, 0.17 mmol, 15.3 mM) and Bu_3SnH (0.63 mL, 2.34 mmol, 0.21 M) in dry PhMe (11 mL) was then injected by syringe over *ca.* 30 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.5 h, TLC control, silica gel, 40:60 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (1.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (15 mL) (Ar atmosphere). After *ca.* 45 min no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 25 cm), using 60:40 EtOAc-hexanes, gave **43.2a** (0.3658 g, 93%) as a pure (^{1}H NMR DMC VIII-111-A), white solid: mp 135-138 °C; $[\alpha]^{25}_D = 21.02°$ (*c* 2.84, $CHCl_3$); FTIR ($CHCl_3$ cast) 1779, 1739, 1664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.82-2.01 [m, 2 H, including a dt at δ 1.88 (J = 12.8, 9.9 Hz)], 2.03-2.12 (m, 1 H), 2.16-2.32 (m, 2 H), 2.48 (ddd, J = 17.4, 9.6, 2.9 Hz, 1 H), 2.62-2.74 (m, 2 H), 2.79 (dt, J = 17.4, 9.8 Hz, 1 H), 3.01 (dd, J = 14.8, 1.3 Hz, 1 H), 4.31-4.38 (m, 1 H), 4.53 (d, J = 8.8 Hz, 1 H), 5.00-5.04 (m, 1 H), 5.05-5.24 [m, 3 H, including an AB q at δ 5.07 and 5.21 ($\Delta\nu_{AB}$ = 55.1 Hz, J = 12.3 Hz)], 7.29-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.36 (t'), 28.54 (t'), 29.04 (t'), 31.03 (t'), 42.19 (t'), 58.14 (d'), 61.80 (d'), 67.11 (t'), 83.04 (s'), 112.03 (t'), 128.19 (d'), 128.42 (d'), 128.60 (d'), 135.42 (s'), 139.07 (s'), 167.30 (s'), 171.01 (s'), 176.39 (s'); exact mass *m/z* calcd for $C_{20}H_{21}NO_5$ 355.1420, found 355.1417.

Phenylmethyl (3'*S*)-Octahydro-8'-methylene-5,5'-dioxospiro[furan-2(3*H*),6'(5'*H*)-indolizine]-3'-carboxylate (43.2b) without isolation of the intermediate stannane.

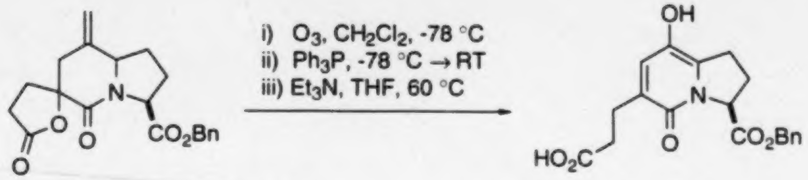


A solution of AIBN (0.0169 g, 0.10 mmol, 7.68 mM) and Bu_3SnH (0.38 mL, 1.41 mmol, 0.11 M) in dry PhMe (13.4 mL) was injected by syringe over *ca.* 1 min into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **42.2b** (0.2360 g, 0.67 mmol) in PhMe (13.4 mL) (Ar atmosphere). Stirring at reflux was continued for 2.5

h. A solution of AIBN (0.0165 g, 0.10 mmol, 15.00 mM) and Bu_3SnH (0.38 mL, 1.41 mmol, 0.21 M) in dry PhMe (6.7 mL) was then injected by syringe over *ca.* 30 sec. Stirring under reflux was continued until no more starting material remained (*ca.* 1.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (1.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (15 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 50:50 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **43.2b** (0.2231 g, 94%) as a pure (^1H NMR), colorless oil: $[\alpha]^{25}_D = -163.35°$ (*c* 1.73, $CHCl_3$); FTIR ($CHCl_3$ cast) 1781, 1745, 1659 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.75 (ddd, *J* = 22.9, 11.7, 7.8 Hz, 1 H), 1.87-2.03 (m, 2 H), 2.22-2.31 (five-line m, 1 H), 2.40-2.58 (m, 3 H), 2.66 (d, *J* = 13.9 Hz, 1 H), 2.87 (dt, *J* = 17.6, 10.6 Hz, 1 H), 3.10 (dd, *J* = 13.9, 1.2 Hz, 1 H), 4.38 (dd, *J* = 10.9, 4.9 Hz, 1 H), 4.56 (t, *J* = 8.6 Hz, 1 H), 5.05 (s, 1 H), 5.12 (d, *J* = 0.6 Hz, 1 H), 5.15 and 5.21 (AB q, $\Delta\nu_{AB}$ = 25.9 Hz, *J* = 12.4 Hz, 2 H), 7.28-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.61 (t'), 28.41 (t'), 30.15 (t'), 32.38 (t'), 42.47 (t'), 58.04 (d'), 61.95 (d'), 67.04 (t'), 82.43 (s'), 113.10 (t'), 128.10 (d'), 128.32 (d'), 128.59 (d'), 135.45 (s'), 138.20 (s'), 167.56 (s'), 171.16 (s'), 176.12 (s'); exact mass *m/z* calcd for $C_{20}H_{21}NO_5$ 355.1420, found 355.1420.

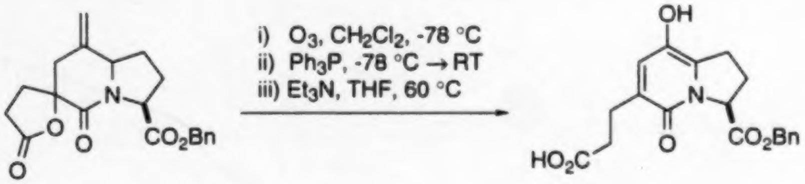
(*S*)-1,2,3,5-Tetrahydro-8-hydroxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoic acid (43.4) from 43.2a.



Freshly distilled CH_2Cl_2 (10 mL) was added to **43.2a** (0.6011 g, 1.69 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 10 min, TLC control, silica gel, 60:40 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.8892 g, 3.39 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product (**43.3a**) could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

Dry Et_3N (1.0 mL, 7.17 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (10 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (2 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **43.4** (0.5746 g, 95%) as a pure (^{1}H NMR DMC VIII-107-A), light-yellow crystals: mp 159-161 °C; $[\alpha]^{25}_D = -158.64°$ (*c* 2.28, MeOH); FTIR ($CHCl_3$ cast) 3450-2400, 1744, 1543 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.26 (ddt, *J* = 13.3, 8.7, 3.3 Hz, 1 H), 2.48-2.63 (m, 3 H), 2.68-2.83 (eight-line m, 2 H), 2.95-3.16 [m, 2 H, including a tt at δ 3.03 (*J* = 17.2, 8.7 Hz), overlapping a ddd at δ 3.11 (*J* = 17.2, 9.5, 3.4 Hz)], 5.13-5.24 [m, 3H, including a dd at δ 5.15 (*J* = 9.7, 3.1 Hz), overlapping an AB q at δ 5.17 and 5.21 ($\Delta\nu_{AB}$ = 13.6 Hz, *J* = 12.3 Hz)], 7.24 (s, 1 H), 7.27-7.38 (m, 5 H); ^{13}C NMR (CD_3OD, 50.3 MHz) δ 26.90 (t'), 27.48 (t'), 28.11 (t'), 33.75 (t'), 63.93 (d'), 68.34 (t'), 129.21 (d'), 129.40 (d'), 129.62 (d'), 129.80 (s'), 133.99 (s'), 134.92 (d'), 137.00 (s'), 137.12 (s'), 160.37 (s'), 171.47 (s'), 176.74 (s'); exact mass *m/z* calcd for $C_{19}H_{19}NO_6$ 357.1213, found 357.1208.

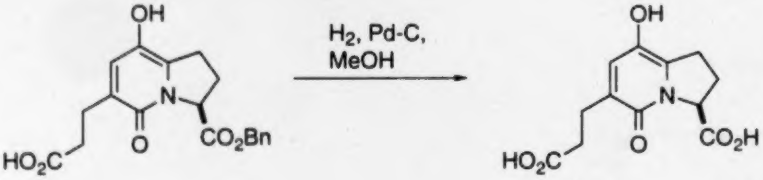
(*S*)-1,2,3,5-Tetrahydro-8-hydroxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoic acid (43.4) from 43.2b.



Freshly distilled CH_2Cl_2 (15 mL) was added to **43.2b** (0.2857 g, 0.80 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 10 min, TLC control, silica gel, 80:20 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.4233 g, 1.61 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow residue; the ketonic product (**43.3b**) could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

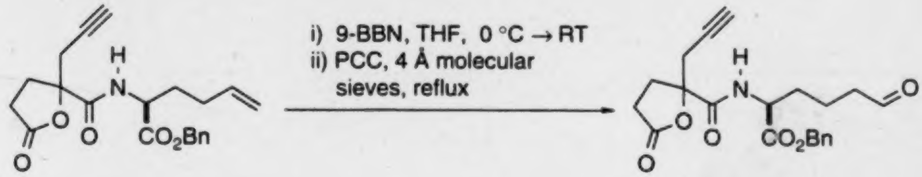
Dry Et_3N (1.5 mL, 10.76 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (15 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (3.5 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **43.4** (0.2758 g, 96%) as a pure (^{1}H NMR), light-yellow oil, spectroscopically identical to material obtained from **43.2a**.

(*S*)-3-Carboxy-1,2,3,5-tetrahydro-8-hydroxy-5-oxo-6-indolizinepropanoic acid (4.1).



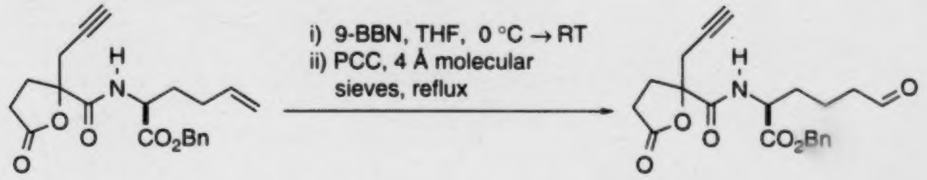
10% Pd-C (*ca.* 25 mg) was added to a stirred solution of **43.4** (0.1614 g, 0.45 mmol) in MeOH (20 mL). The reaction flask was flushed with hydrogen, and the mixture was stirred under hydrogen (balloon) until all of the starting material had been consumed (*ca.* 15 min, TLC control, silica gel, 95:5 EtOAc-AcOH). The mixture was filtered through a sintered glass frit (grade D) and evaporated. Flash chromatography of the residue over reverse phase C-18 silica gel (Toronto Research Chemicals Inc., 10% capped with TMS) (2 x 20 cm), using 90:10 water-MeCN, gave **4.1** (0.1128 g, 93%) as a pure (^{1}H NMR DMC VIII-89-A), white foam: $[\alpha]^{25}_D = -196.55°$ (*c* 0.87, H_2O), lit.[13] $[\alpha]^{25}_D = -199.5°$ (*c* 1.0, H_2O); FTIR (microscope) 3650-2300, 1721 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 2.30 (ddt, *J* = 13.5, 8.4, 3.8 Hz, 1 H), 2.48-2.73 (m, 5 H), 2.95-3.13 [m, 2 H, including a tt at δ 3.03 (*J* = 17.3, 8.8 Hz), overlapping a ddd at δ 3.08 (*J* = 17.3, 9.5, 3.9 Hz)], 5.06 (dd, *J* = 9.9, 3.0 Hz, 1 H), 7.25 (s, 1 H); ^{13}C NMR (D_2O, 75.5 MHz) δ 25.56 (t'), 26.63 (t'), 27.52 (t'), 33.06 (t'), 63.72 (d'), 128.26 (s'), 134.73 (d'), 135.28 (s'), 135.88 (s'), 159.74 (s'), 174.63 (s'), 178.03 (s'); exact mass *m/z* calcd for $C_{12}H_{13}NO_6$ 267.0743, found 267.0735.

6-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-L-norleucine phenylmethyl ester (44.1a).



9-BBN (0.5 M in THF, 1.79 mL, 0.90 mmol) was added by syringe pump over *ca.* 4 min to a stirred and cooled (ice-water bath) solution of **40.2a** (0.2968 g, 0.80 mmol) in dry THF (10 mL) (Ar atmosphere). The cooling bath was removed and stirring was continued for 1 h. The mixture was then transferred by cannula to a stirred and cooled (ice-water bath) suspension of PCC (1.5620 g, 7.25 mmol) and crushed 4 Å molecular sieves (*ca.* 500 mg) in dry CH_2Cl_2 (10 mL). Additional dry CH_2Cl_2 (2 x 1 mL) was used as a rinse. The resulting mixture was transferred to an oil bath and stirred at reflux temperature for 1.5 h, allowed to cool to room temperature, and filtered through a pad (2.5 x 5 cm) of silica gel, using EtOAc as a rinse. Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 80:20 EtOAc-hexanes, gave **44.1a** (0.2539 g, 83%) as a pure (^{1}H NMR DMC IX-47-A), colorless oil: $[\alpha]^{25}_D = -4.36°$ (*c* 1.56, $CHCl_3$); FTIR ($CDCl_3$ cast) 3360, 3288, 2850, 2750, 1788, 1739, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48-1.66 (m, 2 H), 1.67-1.78 (m, 1 H), 1.79-1.94 (m, 1 H), 2.03 (t, *J* = 2.0 Hz, 1 H), 2.34-2.63 (m, 5 H), 2.65-2.83 [m, 3 H, including an apparent dd at δ 2.74 (A of an ABX system, *J* = 17.3, 2.6 Hz), and an apparent dd at δ 2.79 (B of an ABX system, *J* = 17.3, 2.6 Hz)], 4.53 (dt, *J* = 8.1, 5.1 Hz, 1 H), 5.11 and 5.17 (AB q, $\Delta\nu_{AB}$ = 22.4 Hz, *J* = 12.1 Hz, 2 H), 7.11 (d, *J* = 8.1 Hz, 1 H), 7.26-7.39 (m, 5 H), 9.66 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 17.66 (t'), 28.29 (t', two signals overlap), 29.51 (t'), 30.99 (t'), 42.75 (t'), 52.04 (d'), 67.27 (t'), 72.37 (d'), 77.22 (s'), 85.26 (s'), 128.36 (d'), 128.57 (d', two signals overlap), 135.03 (s'), 170.80 (s'), 170.94 (s'), 175.21 (s'), 201.35 (d'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_6$ (M + Na) 408.1423, found 408.1431.

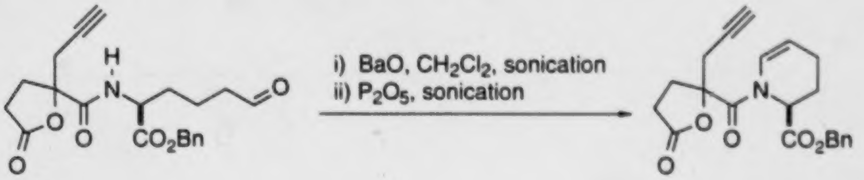
6-Oxo-*N*-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-L-norleucine phenylmethyl ester (44.1b).



9-BBN (0.5 M in THF, 2.83 mL, 1.42 mmol) was added by syringe pump over *ca.* 4 min to a stirred and cooled (ice-water bath) solution of **40.2b** (0.4754 g, 1.29 mmol) in dry THF (10 mL) (Ar atmosphere). The cooling bath was removed and stirring was continued for 1 h. The mixture was then transferred by cannula to a stirred and cooled (ice-water bath) suspension of PCC (2.4908 g, 11.56 mmol) and crushed 4 Å molecular sieves (*ca.* 500 mg) in dry CH_2Cl_2 (10 mL). Additional dry CH_2Cl_2 (2 x 1 mL) was used as a rinse. The resulting mixture was transferred to an oil bath and stirred at reflux temperature for 1.5 h, allowed to cool to room temperature, and filtered through a pad (2.5 x 4 cm) of silica gel, using EtOAc as a rinse. Evaporation of the solvent and flash chromatography of the residue over silica gel (2.5 x 25 cm), using 70:30 EtOAc-hexanes, gave **44.1b** (0.3671 g, 74%) as a pure (^{1}H NMR), colorless oil: $[\alpha]^{25}_D = -1.09°$ (*c* 1.47, $CHCl_3$); FTIR ($CDCl_3$ cast) 3360, 3288, 2840, 2740, 1790, 1739, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.58-1.79 (m, 3 H), 1.89-2.00 (m, 1 H), 2.11 (t, *J* = 2.1 Hz, 1 H), 2.33-2.54 (m, 5 H), 2.60-2.74 (m, 1 H), 2.82 (A of an ABX system, apparent dd, *J* = 17.2, 2.6 Hz, 1 H), 2.86 (B of an ABX system, apparent dd, *J* = 17.2, 2.6 Hz, 1 H), 4.60 (dt, *J* = 8.2, 5.0 Hz, 1 H), 5.11 and 5.20 (AB q, $\Delta\nu_{AB}$ = 35.5 Hz, *J* = 12.1 Hz, 2 H), 6.94 (d, *J* = 8.2 Hz, 1 H), 7.28-7.42 (m, 5 H), 9.71 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ 19.83

(t'), 29.91 (t'), 30.25 (t'), 31.92 (t'), 32.84 (t'), 44.77 (t'), 54.03 (d'), 69.14 (t'), 73.84 (d'), 79.48 (s'), 87.32 (s'), 130.18 (d'), 130.34 (d'), 130.46 (d'), 137.27 (s'), 172.46 (s'), 172.90 (s'), 176.80 (s'), 203.28 (d'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_6$ (M + Na) 408.1423, found 408.1423.

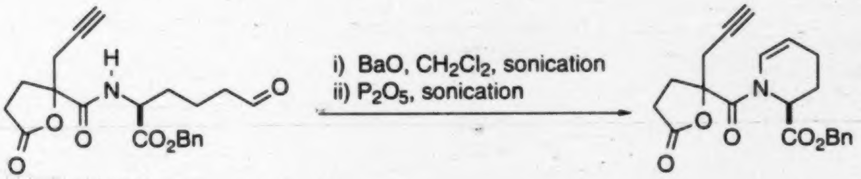
Phenylmethyl (2*S*)-1,2,3,4-Tetrahydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-2-pyridinecarboxylate (44.2a).



BaO (0.1742 g, 1.14 mmol) was tipped into a solution of **44.1a** (0.0879 g, 0.23 mmol) in dry CH_2Cl_2 (5 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.1633 g, 1.15 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 1 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (1.5 x 20 cm), using 40:60 EtOAc-hexanes, gave **44.2a** (0.0704 g, 83%) as a pure (^{1}H NMR DMC IX-49-A), colorless oil, which was a mixture of rotamers: $[\alpha]^{25}_D = -57.77°$ (*c* 1.21, $CHCl_3$); FTIR ($CDCl_3$ cast) 3278, 1792, 1742, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80-2.16 (m, 4 H, including a t at δ 2.09 (*J* = 2.4 Hz), and a t at δ 2.14 (*J* = 2.6 Hz)], 2.24-2.79 (m, 5 H), 2.88-3.10 (m, 2 H), 4.98-5.54 (m, 4 H, including a dd at δ 5.02 (*J* = 8.1, 5.5 Hz), and a br s at δ 5.52)], 7.11-7.22 [m, 1 H, including a d at δ 7.15 (*J* = 8.6

Hz), and a d at δ 7.20 (J = 8.6 Hz)], 7.27-7.42 (m, 5 H); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ 19.06 (t'), 19.19 (t'), 23.70 (t'), 24.35 (t'), 27.76 (t'), 28.21 (t'), 28.73 (t'), 30.41 (t'), 30.70 (t'), 33.06 (t'), 54.20 (d'), 56.59 (d'), 67.43 (t'), 67.86 (t'), 72.47 (d'), 72.87 (d'), 77.12 (s'), 77.73 (s'), 86.64 (s'), 87.80 (s'), 108.64 (d'), 110.81 (d'), 124.58 (d'), 125.24 (d'), 128.46 (d'), 128.71 (d'), 128.89 (d'), 136.12 (s'), 167.93 (s'), 168.10 (s'), 170.23 (s'), 171.02 (s'), 174.95 (s'), 175.42 (s'), not all of the signals from the minor rotamer were observed; exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{21}NNaO_5$ (M + Na) 390.1317, found 390.1325.

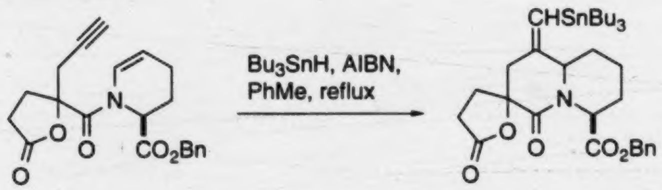
Phenylmethyl (2*S*)-1,2,3,4-Tetrahydro-1-[[tetrahydro-5-oxo-2-(2-propynyl)-2-furanyl]carbonyl]-2-pyridinecarboxylate (44.2b).



BaO (0.3102 g, 2.02 mmol) was tipped into a solution of **44.1b** (0.1536 g, 0.40 mmol) in dry CH_2Cl_2 (10 mL), contained in a round-bottomed flask fused onto a condenser (Ar atmosphere), and the suspension was sonicated (Branson, model B-12, 80 W; Ar atmosphere). Sonication was stopped after 1 h, and P_2O_5 (0.2792 g, 1.97 mmol) was tipped into the flask. The system was re-sealed with a septum and flushed with Ar, and the mixture was sonicated until no more aldehyde remained (*ca.* 1.5 h, TLC control, silica gel, 50:50 EtOAc-hexanes). The suspension was then centrifuged. Evaporation of the supernatant liquid, and flash chromatography of the orange residue over silica gel (1.5 x 20 cm), using 40:60 EtOAc-hexanes, gave **44.2b** (0.1160 g, 80%) as a pure (^{1}H NMR), colorless oil: $[\alpha]^{25}_D$ = -76.63° (c 2.91, $CHCl_3$); FTIR ($CDCl_3$ cast) 3282, 1793, 1742, 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.84-2.15 [m, 4 H, including a t at δ

2.12 (J = 2.4 Hz)], 2.30-2.50 (m, 3 H), 2.62 (ddd, J = 18.1, 9.4, 5.5 Hz, 1 H), 2.84-3.05 [m, 3 H, including an apparent dd at δ 2.88 (A of an ABX system, J = 17.1, 2.5 Hz), and an apparent dd at δ 2.94 (B of an ABX system, J = 17.1, 2.5 Hz)], 5.00-5.06 (m, 1 H), 5.09 and 5.20 (AB q, $\Delta\nu_{AB}$ = 43.3 Hz, J = 12.3 Hz, 2 H), 5.27 (dd, J = 3.3, 3.3 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1 H), 7.24-7.40 (m, 5 H); ^{13}C NMR (CDCl$_3$, 50.3 MHz) δ 18.92 (t'), 23.48 (t'), 27.85 (t'), 28.87 (t'), 30.54 (t'), 53.60 (d'), 67.17 (t'), 72.88 (d'), 76.45 (s'), 86.43 (s'), 109.19 (d'), 124.62 (d'), 128.04 (d'), 128.39 (d'), 128.64 (d'), 135.41 (s'), 166.94 (s'), 169.95 (s'), 175.01 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{21}NNaO_5$ (M + Na) 390.1317, found 390.1320.

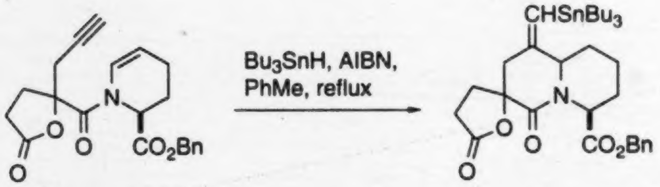
Phenylmethyl(6'*S*)-Octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.1a).



A solution of AIBN (0.0038 g, 0.02 mmol, 7.46 mM) and Bu$_3$SnH (0.09 mL, 0.33 mmol, 0.11 M) in dry PhMe (3.1 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **44.2a** (0.0573 g, 0.16 mmol) in PhMe (3.1 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the solid white residue over silica gel (1.5 x 20 cm), using 30:70 EtOAc-hexanes, gave phenylmethyl (6'*S*)-octahydro-4',5-dioxo-1'-

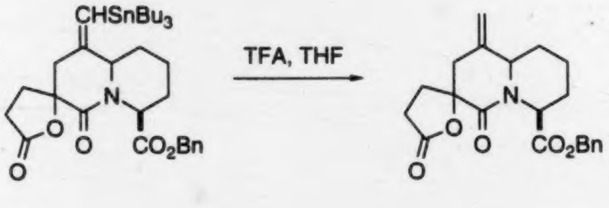
[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (0.0761 g, 75%) as a white, amorphous solid, containing slight impurities (^{1}H NMR): $[\alpha]^{25}_D$ = 17.28° (*c* 1.69, $CHCl_3$); FTIR ($CHCl_3$ cast) 1789, 1744, 1659 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.83-1.05 (m, 15 H), 1.24-2.13 (m, 19 H), 2.40-2.60 (m, 2 H), 2.70-2.93 [m, 3 H, including an AB q at δ 2.75 and 2.79 ($\Delta\nu_{AB}$ = 13.2 Hz, *J* = 13.9 Hz), and a dt at δ 2.86 (*J* = 17.4, 9.5 Hz)], 4.06-4.17 (m, 1 H), 4.22 (dd, *J* = 6.9, 4.6 Hz, 1 H), 5.09 and 5.21 (AB q, $\Delta\nu_{AB}$ = 41.6 Hz, *J* = 12.2 Hz, 2 H), 5.91 (vinyl proton, d, *J* = 0.9 Hz, 1 H, Sn satellite signals at δ 5.84 and 5.98), 7.27-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 10.47 (t'), 13.67 (q'), 17.53 (t'), 19.32 (t'), 24.52 (t'), 27.24 (t'), 28.75 (t'), 29.09 (t'), 31.70 (t'), 42.97 (t'), 57.04 (d'), 60.81 (d'), 66.96 (t'), 82.10 (s'), 127.92 (d'), 128.29 (d'), 128.37 (d'), 128.54 (d'), 135.70 (s'), 145.84 (s'), 169.14 (s'), 170.75 (s'), 176.03 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{33}H_{49}NNaO_5{}^{120}Sn$ (M + Na) 682.2530, found 682.2539.

Phenylmethyl (6'*S*)-Octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.1b).



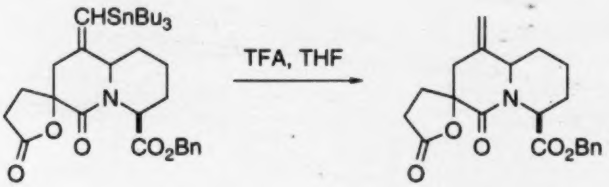
A solution of AIBN (0.0024 g, 0.015 mmol, 7.31 mM) and Bu_3SnH (0.06 mL, 0.22 mmol, 0.11 M) in dry PhMe (2 mL) was injected by syringe over *ca.* 10 sec into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **44.2b** (0.0364 g, 0.10 mmol) in PhMe (2 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation of the solvent and flash chromatography of the solid, white residue over silica gel (1 x 20 cm), using 40:60 EtOAc-hexanes, gave phenylmethyl (6'*S*)-octahydro-4',5-dioxo-1'-[(tributylstannyl)methylene]spiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (0.0511 g, 80%) as a white, amorphous solid, containing slight impurities (^{1}H NMR): $[\alpha]^{25}_D$ = -79.36° (*c* 1.09, $CHCl_3$); FTIR ($CHCl_3$ cast) 1733, 1695, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80-1.07 [m, 15 H, including a t at δ 0.89 (*J* = 7.3 Hz)], 1.31 (sextet, *J* = 7.3 Hz, 6 H), 1.37-1.67 (m, 9 H), 1.75-1.84 (m, 1 H), 1.86-1.98 (m, 2 H), 2.32 (d, *J* = 13.4 Hz, 1 H), 2.44-2.58 (m, 2 H), 2.62-2.71 (m, 1 H), 2.80-2.98 [m, 2 H, including a dt at δ 2.92 (*J* = 17.7, 10.0 Hz)], 4.09 (d, *J* = 12.2 Hz, 1 H), 5.14 and 5.23 (AB q, $\Delta\nu_{AB}$ = 33.9 Hz, *J* = 12.4 Hz, 2 H), 5.48 (dd, *J* = 5.8, 1.7 Hz, 1 H), 5.87 (vinyl proton, d, *J* = 1.0 Hz, 1 H, Sn satellite signals at δ 5.80 and 5.93), 7.25-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 10.48 (t'), 13.64 (q'), 21.03 (t'), 26.06 (t'), 27.22 (t'), 28.74 (t'), 29.06 (t'), 31.90 (t'), 32.34 (t'), 42.91 (t'), 52.55 (d'), 60.78 (d'), 66.98 (t'), 82.01 (s'), 127.83 (d'), 127.96 (d'), 128.27 (d'), 128.61 (d'), 135.47 (s'), 145.62 (s'), 169.04 (s'), 170.20 (s'), 175.94 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{33}H_{49}NNaO_5{}^{120}Sn$ (M + Na) 682.2530, found 682.2533.

Phenylmethyl (6'*S*)-Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.2a).



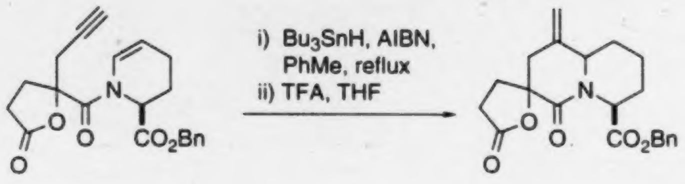
Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the vinyl stannane **45.1a** (0.0803 g, 0.122 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 1 h no more vinyl stannane could be detected (TLC control, silica gel, 30:70 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 50:50 EtOAc-hexanes, gave **45.2a** (0.0433 g, 96%) as a pure (^{1}H NMR), colorless oil, spectroscopically identical to material obtained without isolation of the vinyl stannane.

Phenylmethyl (6'*S*)-Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.2b).



Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the vinyl stannane **45.1b** (0.0366 g, 0.056 mmol) in THF (5 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 40:60 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 60:40 EtOAc-hexanes, gave **45.2b** (0.0204 g, 98%) as a pure (^{1}H NMR), white, amorphous solid, spectroscopically identical to material obtained without isolation of the vinyl stannane.

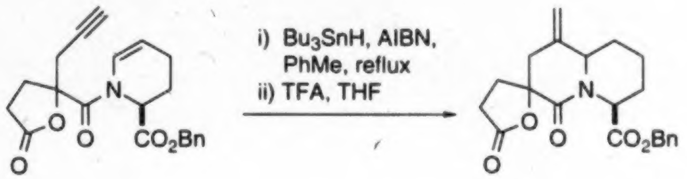
Phenylmethyl (6'*S*)-Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.2a).



A solution of AIBN (0.0083 g, 0.05 mmol, 7.49 mM) and Bu_3SnH (0.19 mL, 0.71 mmol, 0.10 M) in dry PhMe (6.75 mL) was injected by syringe over *ca.* 1 min into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **44.2a** (0.1242 g, 0.34 mmol) in PhMe (6.75 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (5 mL) (Ar atmosphere). After *ca.* 50 min no more vinyl stannane could be detected (TLC control, silica gel, 30:70 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 25 cm), using 50:50 EtOAc-hexanes, gave **45.2a** (0.0924 g, 74%) as a pure (^{1}H NMR DMC IX-97-A), colorless oil: $[\alpha]^{25}{}_D = 0.32°$ (*c* 1.58, $CHCl_3$); FTIR ($CHCl_3$ cast) 1785, 1743, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60-1.82 (m, 3 H), 1.85-2.14 (m, 4 H), 2.39-2.51 (m, 2 H), 2.69 (d, J = 13.4 Hz, 1 H), 2.80 (dt, J = 17.8, 10.6 Hz, 1 H), 2.94 (d, J = 13.4 Hz, 1 H), 4.09 (d, J = 11.8 Hz, 1 H), 4.37 (dd, J = 6.1, 5.0 Hz, 1 H), 5.05-5.25 [m, 4 H, including a d at δ 5.08 (J = 0.6 Hz), a d at δ 5.11 (J = 1.2 Hz), and an AB q at δ 5.09 and 5.22 ($\Delta\nu_{AB}$ = 54.6 Hz, J = 12.2 Hz)], 7.28-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 18.23 (t'), 24.17 (t'), 27.69 (t'), 28.52 (t'), 32.38 (t'), 41.74 (t'), 56.31 (d'), 57.16 (d'), 67.02 (t'), 81.85 (s'), 113.83 (t'), 128.35 (d', two signals overlap), 128.58 (d'), 135.70 (s'), 138.57 (s'), 169.51 (s'), 170.92 (s'), 176.39 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_5$ (M + Na) 392.1474, found 392.1466.

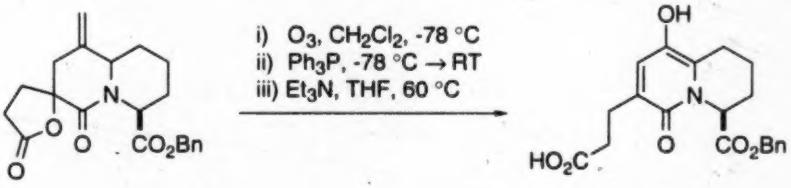
Phenylmethyl (6'*S*)-Octahydro-1'-methylene-4',5-dioxospiro[furan-2(3*H*),3'(4'*H*)-[2*H*]quinolizine]-6'-carboxylate (45.2b).



A solution of AIBN (0.0064 g, 0.04 mmol, 7.79 mM) and Bu_3SnH (0.14 mL, 0.52 mmol, 0.10 M) in dry PhMe (5 mL) was injected by syringe over *ca.* 1 min into a stirred and refluxing solution (0.05 M with respect to the acetylene) of **44.2b** (0.0923 g, 0.25 mmol) in PhMe (5 mL) (Ar atmosphere). Stirring at reflux was continued for 2 h, by which time all of the starting material had been consumed (TLC control, silica gel, 30:70 EtOAc-hexanes), and the mixture was allowed to cool to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave the crude vinyl stannane, which was treated as follows.

Dry CF_3CO_2H (0.5 mL) was injected rapidly into a stirred solution of the above crude vinyl stannane in THF (5 mL) (Ar atmosphere). After *ca.* 30 min no more vinyl stannane could be detected (TLC control, silica gel, 50:50 EtOAc-hexanes). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **45.2b** (0.0851 g, 92%) as a pure (^{1}H NMR), colorless oil: $[\alpha]^{25}_D = -77.63°$ (*c* 2.19, $CHCl_3$); FTIR ($CHCl_3$ cast) 1786, 1737, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32-1.50 (m, 2 H), 1.56-1.69 (m, 1 H), 1.74-1.86 (m, 1 H), 1.90-2.07 (m, 2 H), 2.30-2.42 (m, 2 H), 2.53 (ddd, *J* = 17.7, 9.9, 2.8 Hz, 1 H), 2.62 (d, *J* = 12.9 Hz, 1 H), 2.86 (dt, *J* = 17.7, 10.2 Hz, 1 H), 2.95 (d, *J* = 12.9 Hz, 1 H), 4.10 (d, *J* = 9.6 Hz, 1 H), 5.07 (s, 1 H), 5.10 (s, 1 H), 5.16 and 5.20 (AB q, Δv_{AB} = 17.3 Hz, *J* = 12.4 Hz, 2 H), 5.47 (dd, *J* = 5.7, 1.5 Hz, 1 H), 7.27-7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 20.59 (t'), 26.15 (t'), 28.46 (t'), 30.35 (t'), 32.95 (t'), 41.47 (t'), 52.50 (d'), 57.82 (d'), 67.16 (t'), 81.90 (s'), 113.90 (t'), 128.05 (d'), 128.39 (d'), 128.66 (d'), 135.41 (s'), 138.33 (s'), 169.70 (s'), 170.21 (s'), 176.37 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_5$ (M + Na) 392.1474, found 392.1472.

(*S*)-1,3,4,6-Tetrahydro-9-hydroxy-6-oxo-4-[(phenylmethoxy)carbonyl]-2*H*-quinolizine-7-propanoic acid (45.4) from 45.2a.

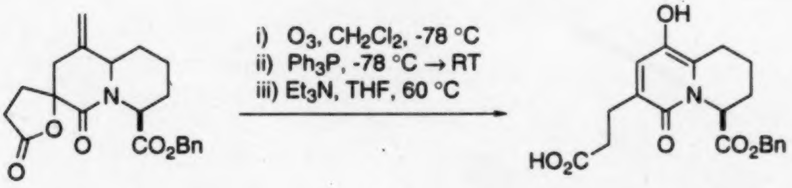


Freshly distilled CH_2Cl_2 (10 mL) was added to **45.2a** (0.0809 g, 0.22 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 7 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.1198 g, 0.46 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

Dry Et_3N (1.0 mL, 7.17 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (10 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (1.5 x 15 cm), using

80:20:5 EtOAc-hexanes-AcOH, gave **45.4** (0.0781 g, 95%) as a pure (^{1}H NMR DMC IX-147-A), light-yellow oil: $[\alpha]^{25}_D = -131.61°$ (*c* 1.18, MeOH); FTIR ($CHCl_3$ cast) 3450-2400, 1743, 1538 cm^{-1}; ^{1}H NMR (CD_3OD, 360 MHz) δ 1.46-1.62 (m, 1 H), 1.69-1.81 (m, 1 H), 2.00-2.13 (m, 1 H), 2.21-2.33 (m, 1 H), 2.49-2.59 (m, 2 H), 2.60-2.82 (m, 3 H), 2.89 (dt, *J* = 18.3, 4.5 Hz, 1 H), 5.09-5.24 [m, 3 H, including an AB q at δ 5.13 and 5.20 ($\Delta\nu_{AB}$ = 26.4 Hz, *J* = 12.3 Hz)], 7.24 (s, 1 H), 7.25-7.37 (m, 5 H); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 16.72 (t'), 23.81 (t'), 26.58 (t'), 27.29 (t'), 33.52 (t'), 57.12 (d'), 68.17 (t'), 127.72 (s'), 129.18 (d'), 129.31 (d'), 129.54 (d'), 130.74 (s'), 132.99 (d'), 137.11 (s'), 138.25 (s'), 161.86 (s'), 172.36 (s', two signals overlap); exact mass (HR electrospray) *m/z* calcd for $C_{20}H_{21}KNO_6$ (M + K) 410.1006, found 410.1001.

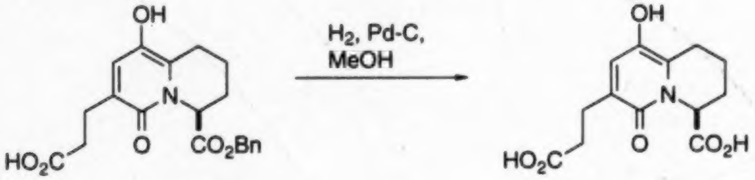
(*S*)-1,3,4,6-Tetrahydro-9-hydroxy-6-oxo-4-[(phenylmethoxy)carbonyl]-2*H*-quinolizine-7-propanoic acid (45.4) from 45.2b.



Freshly distilled CH_2Cl_2 (10 mL) was added to **45.2b** (0.1008 g, 0.27 mmol) contained in a three-necked flask closed by a stopper and fitted with a condenser (not attached to a water supply) closed by a drying tube packed with Drierite, and an ozone-oxygen inlet. The resulting solution was stirred and cooled (-78 °C), and ozone was then bubbled through the solution until all of the starting material had been consumed (*ca.* 6 min, TLC control, silica gel, 50:50 EtOAc-hexanes). The solution was purged with oxygen for 10 min, and then Ph_3P (0.1432 g, 0.55 mmol) was added. The cooling bath was removed and stirring was continued for 1.5 h, by which time the mixture had warmed to room temperature. Evaporation (<0.1 mm Hg) of the solvent gave a light-yellow solid; the ketonic product could not be separated chromatographically from Ph_3PO, and so the crude mixture was used directly.

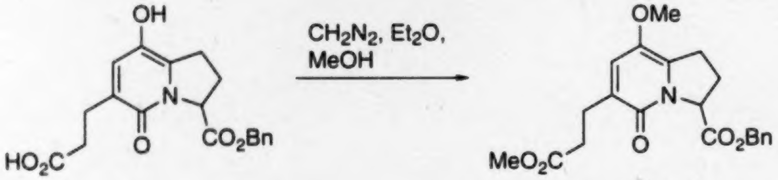
Dry Et_3N (1.0 mL, 7.17 mmol) was added to a stirred solution of the above crude ozonolysis product in dry THF (10 mL) (Ar atmosphere). Stirring was continued at 60 °C (oil bath) for 1.5 h, and the mixture was then cooled and evaporated. Flash chromatography of the light-yellow oily residue over silica gel (1.5 x 20 cm), using 80:20:5 EtOAc-hexanes-AcOH, gave **45.4** (0.0963 g, 96%) as a pure (^1H NMR), light-yellow foam, spectroscopically identical to material obtained from **45.2a**.

(*S*)-4-Carboxy-1,3,4,6-tetrahydro-9-hydroxy-6-oxo-2*H*-quinolizine-7-propanoic acid (4.2).



10% Pd-C (*ca.* 25 mg) was added to a stirred solution of **45.4** (0.0565 g, 0.152 mmol) in MeOH (5 mL). The reaction flask was flushed with hydrogen, and the mixture was stirred under hydrogen (balloon) until all of the starting material had been consumed (*ca.* 20 min, TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). The mixture was filtered through a sintered glass frit (grade D) and evaporated. Flash chromatography of the residue over reverse phase C-18 silica gel (Toronto Research Chemicals Inc., 10% capped with TMS) (1 x 20 cm), using 90:10 water-MeCN, gave **4.2** (0.0411 g, 96%) as a pure (^1H NMR DMC IX-127-A), white foam: $[\alpha]^{25}_D = -139.82°$ (*c* 1.67, H_2O), lit.[13] $[\alpha]^{25}_D = -141.2°$ (*c* 0.16, H_2O); FTIR ($CHCl_3$-MeOH cast) 3525-2375, 1723 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 1.58-1.72 (m, 1 H), 1.78-1.90 (m, 1 H), 2.08-2.20 (m, 1 H), 2.27-2.38 (m, 1 H), 2.59-2.82 (m, 5 H), 2.91 (dt, J = 18.2, 4.9 Hz, 1 H), 5.11 (dd, J = 6.3, 4.0 Hz, 1 H), 7.35 (s, 1 H); ^{13}C NMR (D_2O, 50.3 MHz) δ 15.83 (t'), 23.43 (t'), 25.79 (t'), 26.17 (t'), 33.07 (t'), 57.30 (d'), 126.53 (s'), 132.35 (s'), 133.12 (d'), 137.43 (s'), 161.50 (s'), 175.85 (s'), 178.26 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{13}H_{16}NO_6$ (M + H) 282.0978, found 282.0976.

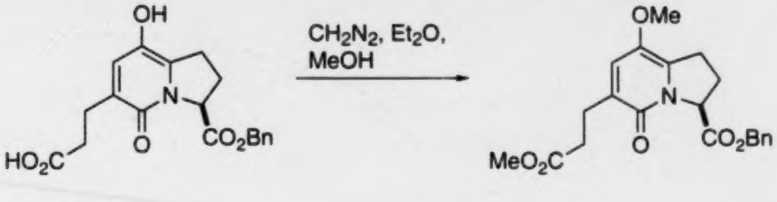
Methyl 1,2,3,5-Tetrahydro-8-methoxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoate (46.1).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **37.4** (0.0183 g, 0.051 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). The mixture was evaporated under reduced pressure to give a yellow residue. Flash chromatography of the residue over silica gel (1 x 15 cm), using 80:20 EtOAc-hexanes, gave **46.1** (0.0156 g, 78%) as a pure (^{1}H NMR), light-yellow oil: FTIR ($CDCl_3$ cast) 1739, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.21-2.31 (m, 1 H), 2.47 (ddd, J = 18.8, 13.4, 9.4 Hz, 1 H), 2.60-2.73 (m, 2 H), 2.76-2.93 (m, 2 H), 3.03-3.14 (m, 2 H), 3.65 (s, 3 H), 3.73 (s, 3 H), 5.12-5.30 [m, 3 H, including a dd at δ 5.16 (J = 9.5, 3.3 Hz), and an AB q at δ 5.17 and 5.27 ($\Delta\nu_{AB}$ = 38.6 Hz, J = 12.3 Hz)], 7.27 (s, 1 H), 7.29-7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 26.41 (t'), 26.59 (t'), 27.44 (t'), 32.52 (t'), 51.53 (q'), 59.01 (q'), 62.00 (d'), 67.41 (t'), 128.19 (d'), 128.42 (d'), 128.63 (d'), 129.37 (s'), 131.46 (d'), 135.35 (s'), 135.89 (s'), 137.54 (s'), 159.17 (s'), 170.00 (s'), 173.64 (s'); exact mass

(HR electrospray) *m/z* calcd for $C_{21}H_{24}NNaO_6$ (M + H) 386.1604, found 386.1611.

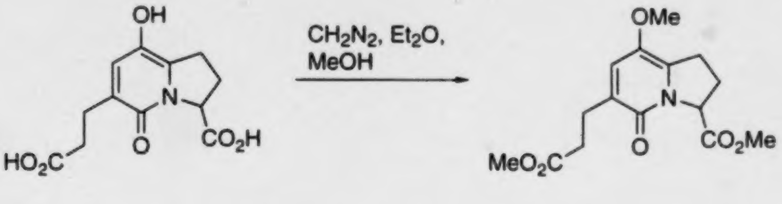
Methyl (*S*)-1,2,3,5-Tetrahydro-8-methoxy-5-oxo-3-[(phenylmethoxy)carbonyl]-6-indolizinepropanoate (46.2).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **43.4** (0.0342 g, 0.096 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **46.2** (0.0277 g, 75%) as a pure (^{1}H NMR DMC IX-141-A), light-yellow oil: $[\alpha]^{25}_D = -165.45°$ (*c* 1.32, $CHCl_3$); FTIR ($CDCl_3$ cast) 1739, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.20-2.31 (m, 1 H), 2.47 (ddd, *J* = 18.8, 13.4, 9.4 Hz, 1 H), 2.26-2.70 (m, 2 H), 2.75-2.93 (m, 2 H), 3.03-3.12 (m, 2 H), 3.64 (s, 3 H), 3.72 (s, 3 H), 5.11-5.30 [m, 3 H, including a dd at δ 5.15 (*J* = 9.5, 3.3 Hz), overlapping an AB q at δ 5.17 and 5.26 ($\Delta\nu_{AB}$ = 34.6 Hz, *J* = 12.4 Hz)], 7.26 (s, 1 H), 7.27-7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 26.37 (t'), 26.54 (t'), 27.40 (t'), 32.47 (t'), 51.52 (q'), 58.96 (q'), 61.96 (d'), 67.38 (t'), 128.17 (d'), 128.41 (d'), 128.61 (d'), 129.31 (s'), 131.42 (d'), 135.30 (s'), 135.86 (s'), 137.51 (s'), 159.15 (s'), 170.00 (s'), 173.64 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{21}H_{23}NNaO_6$ (M + Na) 408.1423, found 408.1432.

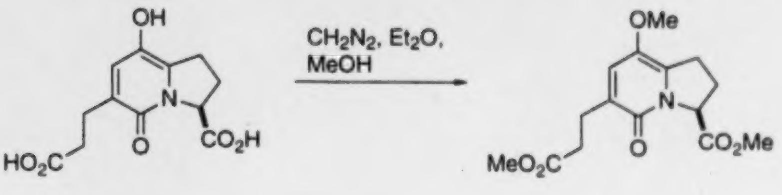
HPLC analysis [Chiralcel OD-H (0.46 x 25 cm), 15% EtOH in hexane] of the above material, and comparison with the corresponding racemic compound, indicated an ee of 99.5%.

Methyl 1,2,3,5-Tetrahydro-8-methoxy-3-(methoxycarbonyl)-5-oxo-6-indolizinepropanoate (46.3).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **4.1** (0.0246 g, 0.092 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 95:5 EtOAc-MeOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 15 cm), using 95:5 EtOAc-MeOH, gave **46.3** (0.0202 g, 71%) as a pure (^{1}H NMR), light-yellow oil: FTIR ($CDCl_3$ cast) 1739, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.22-2.33 (m, 1 H), 2.49 (ddd, *J* = 18.6, 13.4, 9.3 Hz, 1 H), 2.61-2.69 (m, 2 H), 2.73-2.92 (m, 2 H), 3.04-3.15 (m, 2 H), 3.64 (s, 3 H), 3.73 (s, 3 H), 3.78 (s, 3H), 5.09 (dd, *J* = 9.5, 3.5 Hz, 1 H), 7.26 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 26.37 (t'), 26.63 (t'), 27.45 (t'), 32.45 (t'), 51.50 (q'), 52.72 (q'), 58.96 (q'), 61.88 (d'), 129.30 (s'), 131.39 (d'), 135.86 (s'), 137.56 (s'), 159.12 (s'), 170.68 (s'), 173.63 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{19}NNaO_6$ (M + Na) 332.1110, found 332.1110.

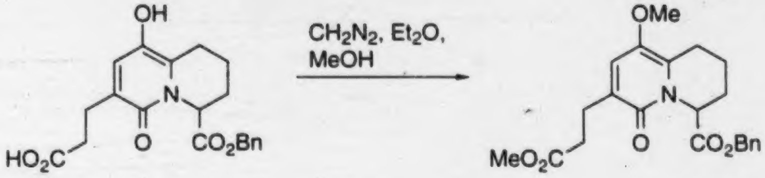
Methyl (*S*)-1,2,3,5-Tetrahydro-8-methoxy-3-(methoxycarbonyl)-5-oxo-6-indolizinepropanoate (46.4).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **4.1** (0.0835 g, 0.31 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 95:5 EtOAc-MeOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 95:5 EtOAc-MeOH, gave **46.4** (0.0705 g, 74%) as a pure (^{1}H NMR DMC IX-145-A), light-yellow oil: $[\alpha]^{25}_D$ = -158.37° (*c* 4.54, $CHCl_3$); FTIR ($CDCl_3$ cast) 1739, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.21-2.31 (m, 1 H), 2.47 (ddd, *J* = 18.6, 13.4, 9.4 Hz, 1 H), 2.60-2.67 (m, 2 H), 2.71-2.90 (m, 2 H), 3.04-3.12 (m, 2 H), 3.61 (s, 3 H), 3.71 (s, 3 H), 3.76 (s, 3 H), 5.07 (dd, *J* = 9.5, 3.4 Hz, 1 H), 7.24 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 26.30 (t'), 26.56 (t'), 27.39 (t'), 32.38 (t'), 51.46 (q'), 52.67 (q'), 58.88 (q'), 61.86 (d'), 129.19 (s'), 131.35 (d'), 135.87 (s'), 137.56 (s'), 158.99 (s'), 170.61 (s'), 173.55 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{19}NNaO_6$ (M + Na) 332.1110, found 332.1112.

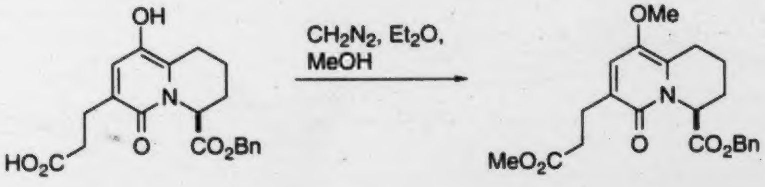
HPLC analysis [Chiralpak AS (0.46 x 25 cm), 10% EtOH in hexane] of the above material, and comparison with the corresponding racemic compound (clear baseline resolution of the enantiomers was not obtained), indicated an ee of 96.2%.

Methyl 1,3,4,6-Tetrahydro-9-methoxy-6-oxo-4-[(phenylmethoxy)carbonyl]-2*H*-quinolizine-7-propanoate (47.1).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **39.4** (0.0415 g, 0.112 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). The mixture was evaporated under reduced pressure to give a yellow residue. Flash chromatography of the residue over silica gel (1.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **47.1** (0.0362 g, 81%) as a pure (^{1}H NMR), light-yellow oil: FTIR ($CHCl_3$ cast) 1739 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.51-1.67 (m, 1 H), 1.68-1.79 (m, 1 H), 1.94-2.08 (m, 1 H), 2.24-2.35 (m, 1 H), 2.58-2.72 (m, 3 H), 2.74-2.95 (m, 3 H), 3.64 (s, 3 H), 3.70 (s, 3 H), 5.09-5.30 [m, 3 H, including an AB q at δ 5.12 and 5.26 ($\Delta\nu_{AB}$ = 51.2 Hz, J = 12.4 Hz)], 7.26 (s, 1 H), 7.28-7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 15.83 (t'), 22.69 (t'), 25.55 (t'), 26.80 (t'), 32.38 (t'), 51.50 (q'), 55.14 (q'), 58.80 (d'), 67.11 (t'), 127.24 (s'), 128.10 (d'), 128.26 (d'), 128.52 (d'), 129.88 (s'), 133.05 (d'), 135.63 (s'), 138.76 (s'), 160.72 (s'), 170.80 (s'), 173.70 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{22}H_{25}NNaO_6$ (M + Na) 422.1580, found 422.1584.

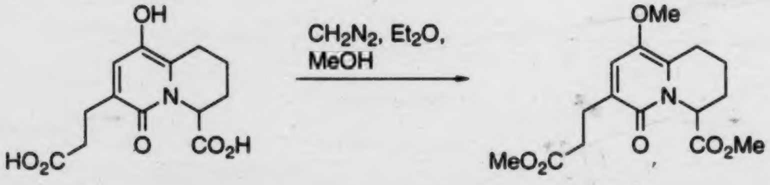
Methyl (*S*)-1,3,4,6-Tetrahydro-9-methoxy-6-oxo-4-[(phenylmethoxy)carbonyl]-2*H*-quinolizine-7-propanoate (47.2).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **45.4** (0.0236 g, 0.064 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 80:20:5 EtOAc-hexanes-AcOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (1 x 15 cm), using 80:20 EtOAc-hexanes, gave **47.2** (0.0201 g, 78%) as a pure (^{1}H NMR), light-yellow oil: $[\alpha]^{25}_{D} = -124.70°$ (*c* 1.66, $CHCl_3$); FTIR ($CHCl_3$ cast) 1739 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53-1.67 (m, 1 H), 1.68-1.80 (m, 1 H), 1.95-2.08 (m, 1 H), 2.24-2.36 (m, 1 H), 2.58-2.72 (m, 3 H), 2.75-2.95 (m, 3 H), 3.64 (s, 3 H), 3.71 (s, 3 H), 5.09-5.30 [m, 3 H, including an AB q at δ 5.13 and 5.27 ($\Delta\nu_{AB}$ = 57.6 Hz, *J* = 12.4 Hz)], 7.27 (s, 1 H), 7.28-7.38 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 15.88 (t'), 22.71 (t'), 25.58 (t'), 26.83 (t'), 32.41 (t'), 51.51 (q'), 55.16 (q'), 58.82 (d'), 67.13 (t'), 127.27 (s'), 128.11 (d'), 128.27 (d'), 128.54 (d'), 129.89 (d'), 133.06 (s'), 135.66 (s'), 138.77 (s'), 160.75 (s'), 170.82 (s'), 173.71 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{22}H_{26}NO_6$ (M + H) 400.1760, found 400.1765.

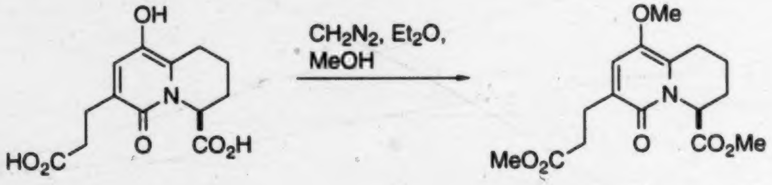
HPLC analysis [Chiralcel OD-H (0.46 x 15 cm), 15% EtOH in hexane] of the above material, and comparison with the corresponding racemic compound, indicated an ee of 99.6%.

Methyl 1,3,4,6-Tetrahydro-9-methoxy-4-(methoxycarbonyl)-6-oxo-2*H*-quinolizine-7-propanoate (47.3).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **4.2** (0.0254 g, 0.090 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 95:5 EtOAc-MeOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 15 cm), using 95:5 EtOAc-MeOH, gave **47.3** (0.0216 g, 74%) as a pure (^{1}H NMR), light-yellow oil: FTIR ($CHCl_3$ cast) 1739 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.59-1.82 (m, 2 H), 1.96-2.08 (m, 1 H), 2.24-2.34 (m, 1 H), 2.60-2.81 (m, 4 H), 2.82-2.96 (m, 2 H), 3.63 (s, 3 H), 3.71 (s, 3 H), 3.75 (s, 3 H), 5.15 (dd, *J* = 6.5, 3.6 Hz, 1 H), 7.26 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 15.88 (t'), 22.73 (t'), 25.61 (t'), 26.79 (t'), 32.34 (t'), 51.47 (q'), 52.52 (q'), 55.13 (q'), 58.77 (d'), 127.18 (s'), 129.82 (d'), 133.04 (s'), 138.76 (s'), 160.68 (s'), 171.47 (s'), 173.68 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{16}H_{21}NNaO_6$ (M + Na) 346.1267, found 346.1266.

Methyl (*S*)-1,3,4,6-Tetrahydro-9-methoxy-4-(methoxycarbonyl)-6-oxo-2*H*-quinolizine-7-propanoate (47.4).



An excess of ethereal CH_2N_2 was added to a stirred and cooled (ice-water bath) solution of **4.2** (0.0312 g, 0.111 mmol) in MeOH (10 mL). The cooling bath was removed and the solution was stirred for 4 h, by which time all of the starting material had been consumed (TLC control, silica gel, 95:5 EtOAc-MeOH). Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 x 15 cm), using 95:5 EtOAc-MeOH, gave **47.4** (0.0258 g, 72%) as a pure (^{1}H NMR), light-yellow oil: $[\alpha]^{25}_D$ =

-101.42° (*c* 2.47, $CHCl_3$); FTIR ($CDCl_3$ cast) 1739 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.59-1.82 (m, 2 H), 1.96-2.08 (m, 1 H), 2.24-2.34 (m, 1 H), 2.60-2.96 (m, 6 H), 3.63 (s, 3 H), 3.71 (s, 3 H), 3.75 (s, 3 H), 5.16 (dd, *J* = 6.5, 3.6 Hz, 1 H), 7.26 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 15.90 (t'), 22.75 (t'), 25.62 (t'), 26.82 (t'), 32.35 (t'), 51.47 (q'), 52.53 (q'), 55.13 (q'), 58.77 (d'), 127.20 (s'), 129.84 (d'), 133.05 (s'), 138.78 (s'), 160.69 (s'), 171.48 (s'), 173.68 (s'); exact mass (HR electrospray) *m/z* calcd for $C_{16}H_{21}NNaO_6$ (M + Na) 346.1267, found 346.1260.

HPLC analysis [Chiralpak AS (0.46 x 25 cm), 10% EtOH in hexane] of the above material, and comparison with the corresponding racemic compound, indicated an ee of 98.3%.

6 REFERENCES AND FOOTNOTES

1) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 7th Ed.; Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, W. R., Eds.; Macmillan: London, 1985; p 639.

2) Page, I. H.; Bumpuss, F. M. *Handbuch der Experimentellen Pharmakologie* **1974**, *37*.

3) Regoli, D. *Pharmacol. Rev.* **1974**, *26*, 69.

4) Streeten, D. H. P; Anderson, G. H. In *Handbook of Hypertension, Angiotensin-Receptor Blocking Drugs*, Doyle, A. E., Ed.; Elsevier Science Publishers, 1984, 5: p246.

5) Carini, D. J.; Duncia, J. V. *J. Med. Chem.* **1991**, *34*, 2525.

6) Chang, R. S. L.; Lotti, V. *Mol. Pharmacol.* **1991**, *29*, 347.

7) Schambye, H. T.; Hjorth, S. A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7046.

8) Ferguson, R. K.; Vlasses, P. H. *J. Clin. Pharmacol.* **1982**, *22*, 281.

9) Cushman, D. W.; Ondetti, M. A. *Biochem.* **1977**, *16*, 5484.

10) Gross, D. M.; Sweet, C. S. *J. Pharmacol. Exp. Ther.* **1981**, *216*, 552.

11) Mynderse, J. S.; Samlaska, S. K.; Fukuda, D. S.; Du Bus, P. J.; Baker, P. J. *J. Antibiot.* **1985**, *38*, 1003.

12) O'Connor, S.; Somers, P. *J. Antibiot.* **1985**, *38*, 993.

13) Hunt, A. H.; Mynderse, J. S.; Samlaska, S. K.; Fukuda, D. S.; Maciak, G. M. *J. Antibiot.* **1988**, *41*, 771.

14) Nakatsukasa, W. M.; Wilgus, R. M.; Thomas, D. D.; Mertz, F. P.; Boeck, L. V. D. *J. Antibiot.* **1985**, *38*, 997.

15) Fang, F. G.; Danishefsky, S. J. *Tetrahedron Lett.* **1989**, *30*, 3621.

16) Wong, P. L.; Moeller, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 11434.

17) Straub, C. S.; Padawa, A. *Org. Lett.* **1999**, *1*, 83.

18) de Lima, D. P., Ph.D. Disseration, Universidade Federal Minas Gerais, Brazil, 1994.

19) Zhou, Y., Ph.D. Disseration, University of Alberta, Alberta, 1997.

20) (a) Bhandal, H.; Pattenden, G. *J. Chem. Soc., Chem. Commun.* **1988**, 1110. (b) Bandaranayake, W. M.; Pattenden, G. *J. Chem. Soc., Chem. Commun.* **1988**, 1179. (c) Bhandal, H.; Pattenden, G., *J. Chem. Soc., Perkin Trans. 1* **1990**, 2709. (d) Summers, M. F.; Marzilli, L. G; Randaccio, L. *J. Am. Chem. Soc.* **1984**, *106*, 4478. (e) Gill, G. B.; Pattenden, G.; Reynolds, S. J. *Tetrahedron Lett.* **1989**, *30*, 3229. (f) Pattenden G.; Howell, A. R. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2715. (g) Pattenden G.; Harrowven, D. C.; Ali, A. *Tetrahedron Lett.* **1992**, *33*, 2851.

21) (a) Pattenden G.; Patel, V. F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2703. (b) Pattenden G.; Patel, V. F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2691.

22) Bigotto, A.; Costa, G.; Mestroni, G.; Pellizer, G.; Pexeddu, A.; Tauzha, G. *Inorg. Chem. Acta Rev.* **1970**, *4*, 41.

23) (a) Akane, N.; Hatano, T.; Ishii, Y. *J. Org. Chem.* **1994** *59*, 7902. (b) Molander, G. A.; Etter, J. B. *J. Org. Chem.* **1986**, *51*, 1778. (c) Takai, K.; Nitta, K.;

Fujimura, O.; Utimoto, K. *J. Org. Chem.* **1989**, *54*, 4732. (d) Hodgson, D. M. *J. Organomet. Chem.* **1994**, *476*, 1.

24) (a) Yuasa, Y.; Kano, S.; Shibuya, S. *Heterocycles* **1991**, *32*, 2311. (b) Beckwith, A. L. J.; Joseph, S. P.; Mayadunne, R. T. A. *J. Org. Chem.* **1993**, *58*, 4198. (c) Beckwith, A. L. J.; Westwood, S. W. *Tetrahedron* **1989**, *45*, 5269.

25) (a) Läuger, P.; Prost, M.; Charlier, R. *Helv. Chim. Acta* **1959**, *42*, 2379. (b) Schneider, D. F.; Weedon, B. C. L. *J. Chem. Soc. (C)* **1967**, 1686. (c) Janardhanam, S.; Shanmugam, P.; Rajagopalan, K. *J. Org. Chem.* **1993**, *58*, 7782. (d) Janardhanam, S.; Balakumar, A.; Rajagopalan, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 551.

26) *Cf.* Klein, J. In *The Chemistry of the Carbon-Carbon Triple Bond, Part 1*; Patai, S., Ed.; Wiley: Chichester, 1978; p 343.

27) Dunn, M. J.; Jackson, R. F. W. *J. Chem. Soc., Chem. Commun.* **1992**, 319.

28) Yeh, M. C. P.; Knochel, P. *Tetrahedron Lett.* **1989**, *30*, 4799.

29) Dunn, M. J.; Jackson, R. F. W.; Pietruszka, J.; Wishart, N.; Ellis, D.; Wythes, M. J. *Synlett* **1993**, 499.

30) The Lemieux-Johnson procedure gave, in this instance, poorer yields.

31) *Cf.* Paterson, I.; Cowden, C.; Watson, C. *Synlett* **1996**, 209.

32) Brown, C. A.; Coleman, R. A. *J. Org. Chem.* **1979**, *44*, 2328.

33) *Cf.* Brown, H. C.; Kulkarni, S. U.; Rao, C. G. *Synthesis*, **1980**, 151.

34) Oxidation of the boranes to the aldehydes could also be done with TPAP, but in lower yield.

35) The reported $[\alpha]^{25}_D = -199.5°$ (c 1.0, H_2O) (see reference 13).

36) The reported $[\alpha]^{25}_D = -141.2°$ (c 0.16, H_2O) (see reference 13); our material had $[\alpha]^{25}_D = -139.82°$ (c 1.67, H_2O).

37) The HPLC analyses were conducted by Dr. P. L. Beaulieu and D. Tung at Bio-Méga Boehringer Ingelheim (Laval, Quebec).

38) Gawley, R. E.; Aubé, J. *Principles of Asymmetric Synthesis*; Elsevier: New York, 1996, p.65.

39) Supplied by Chemical Dynamics Corp., South Plainfield, N. J.

40) Phosphomolybdic acid (15g) and $(NH_4)_2Ce(NO_3)_6$ (2.5g) dissolved in a mixture of water (485 mL) and concentrated H_2SO_4 (15 mL).

41) The compound can also be prepared by using diazomethane: Hagen, T. J.; Narayanan, K.; Names, J.; Cook, J. M. *J. Org. Chem.* **1989**, *54*, 2170.

42) *Cf.* Gibson, S. E.; Gibson, V. C.; Keen, S. P. *Chem. Commun.* **1997**, 1107.

43) Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. *J. Org. Chem.* **1992**, *57*, 3397.

CHAPTER 2

STUDIES ON PEPTIDE SEGMENT COUPLING BY PRIOR LIGATION AND PROXIMITY-INDUCED INTRAMOLECULAR ACYL TRANSFER

1 INTRODUCTION

1.1 Background

Central to the understanding and use of peptides is the extent to which one can manipulate the primary sequence. Complete control — through synthesis — of the primary sequence of a peptide of up to 50 amino acid residues is possible using chemically-based techniques.[1] For larger peptides, however, chemical synthesis is a much more arduous task and, in these cases, site-directed mutagenesis[2] is the approach normally used to modify the primary sequence. This technique is not without its shortcomings since, with few exceptions, only naturally-occurring amino acids can be incorporated into the peptide chain. To some extent, this restriction can be avoided by biosynthetic site-specific incorporation[3] of unnatural amino acids, but this and site-directed mutagenesis are typically used to alter only the sequence of an existing peptide, and so do not offer ready access to peptides that have no known, naturally-occurring counterpart. Therefore, the development of purely chemical synthetic procedures would provide a valuable complementary method for controlling the primary sequence of peptides. An approach based on chemical synthesis would offer an opportunity to incorporate *directly* non-genetically coded amino acids, as well as structural and functional modifications of the peptide backbone and/or its side-chains, and would allow linking of discrete protein domains.

Of fundamental importance to the synthesis of peptides by chemical methods is the formation of peptide bonds between the constituent amino acid residues. Peptide bond formation was reported in 1881 by Curtius,[4] and later (1902) by Fischer.[5] The process typically relies on enthalpic activation of the α-carboxyl group of one amino acid, which then undergoes nucleophilic attack by the α-amino group of a second amino acid. In order to avoid side reactions that might result from the presence of electrophilic and nucleophilic centers in the amino acid side-chains, protecting groups are usually employed. The application of these basic principles to peptide synthesis in the solid phase was reported by Merrifield in 1963.[6] His method has undergone many improvements,[7-10] and it is currently possible to carry out routine stepwise solid phase synthesis of peptides of up to about 50 amino acids.[11] The practicality of solid phase synthesis of peptides greater than about 50 residues in length is marred by the accumulation of byproducts that result during each cycle of the synthetic sequence; these byproducts eventually increase to a level that renders purification of substantial amounts of the desired product difficult.[7,8]

Chemical synthesis of large peptides by intermolecular coupling of smaller, pre-assembled peptides, using conventional peptide bond-forming techniques has been studied.[12] Conceptually, an approach of this type would combine the power of solid phase peptide synthesis to generate moderately-sized segments with a technique that allows rapid assembly of the segments into a large peptide. Based on the ease of discriminating between the relatively small starting materials and the products, it should also be easier to purify and characterize intermediates after each peptide bond-forming step. In practice, however, these potential advantages are not fully realized, and such intermolecular segment couplings have proven unsatisfactory.[8]

The above approach relies ultimately on coupling between the free α-amino group of one peptide segment and the activated α-carboxyl of another. Recently, considerable progress has been made by use of a less conventional procedure for generating a peptide bond between two segments. The approach relies on highly specific and efficient ligation of two peptide segments prior to formation of the peptide bond that will link them in the desired way. Once the two subunits have been ligated, the carboxyl terminus of one and the amino terminus of the other are close together, so that a highly efficient, proximity-driven intramolecular acyl transfer can occur to form a new peptide bond.

Advances in the area of peptide segment coupling that utilize this novel approach have been the result mainly of the independent research carried out by the groups of Kemp, Kent, and Tam. Although their approaches share the common features of prior ligation and intramolecular acyl transfer, they each employ a different method to incorporate these features.

The present review describes these approaches to peptide segment coupling, and is limited to those methods that involve *both prior ligation and intramolecular acyl transfer.*

1.2 Significance of Prior Ligation and Proximity-Induced Intramolecular Acyl Transfer

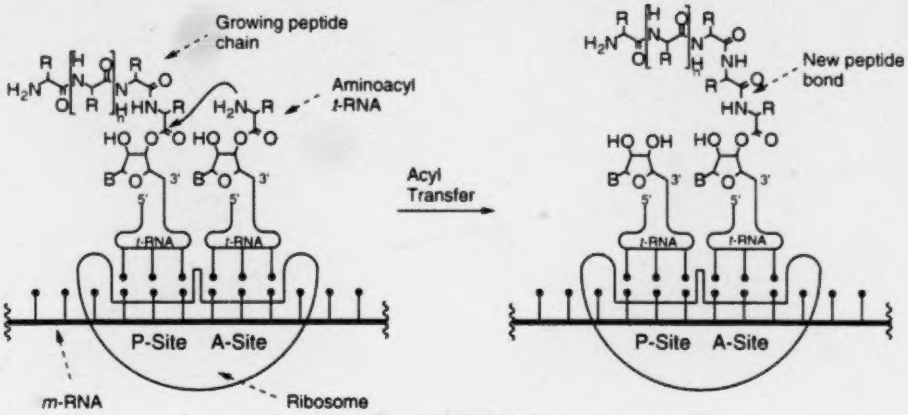
It has long been recognized that intramolecular reactions often occur much more readily than corresponding intermolecular processes.[13] The exact source of the rate acceleration is controversial,[14] but it appears to result largely from the fact that the reacting functional groups are held close together, thereby increasing their effective local concentration.

Peptide bond formation by ***intramolecular*** acyl transfer was demonstrated by Wieland in the early 1950s,[15] and shortly afterwards by Brenner.[16] The concept of entropic activation, as well as its significance as it pertains to peptide bond formation by intramolecular acyl transfer, was first formally described by Brenner in a publication[17] that adumbrated much of the future developments in the area of peptide segment coupling via intramolecular acyl transfer, and briefly mentioned the prior ligation aspect of this approach.

Nature takes advantage of entropic proximity effects in the formation of peptide bonds during protein synthesis. *In vivo* peptide bond formation occurs when the *C*-terminal acyl group of a growing peptide chain is transferred to the α-amino group of an aminoacyl *t*-RNA (**1.1** → **1.2**, Scheme 1). Prior to the transfer the *C*-terminal acyl group and the α-amino group of the aminoacyl *t*-RNA are held, through a complex series of associations, in such a way that they are in proximity. Thus, acyl transfer can proceed despite the fact that the acyl group is in the form of an ordinary ester (linked to a *t*-RNA molecule) and, therefore, has no significant enthalpic activation. This description is of course an oversimplification of the actual cellular processes involved.

It is reasonable to assume that proximity effects also play a role during the *in vivo* post-translational splicing[18] of proteins. In this type of process, an intervening peptide

Scheme 1ª



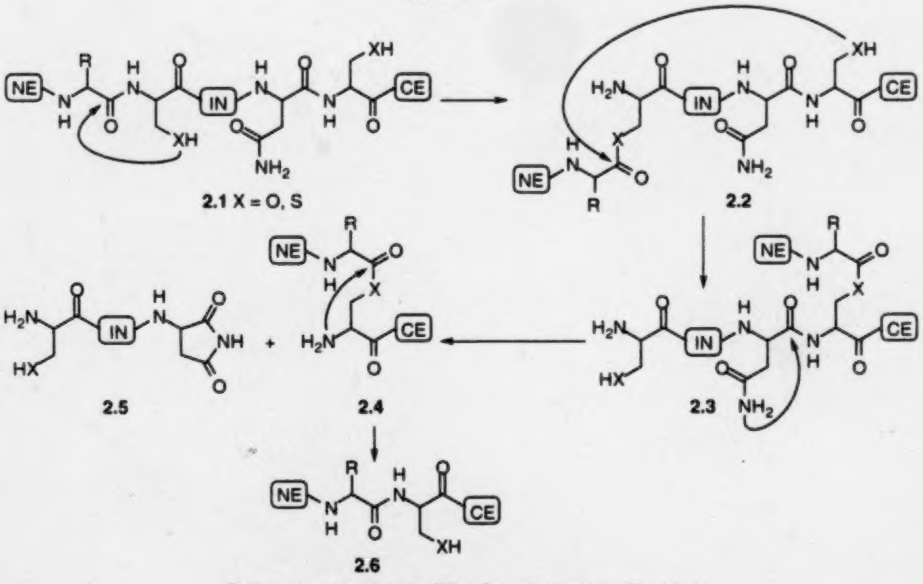
1.1

1.2

ªB = nucleotide base

segment (the intein) is excised while its flanking regions (the exteins) are coupled by formation of a new peptide bond.[18] The sequence is initiated (Scheme 2) by an intramolecular *N*→*O* or *N*→*S* acyl transfer (**2.1** → **2.2**) which gives an intermediate

Scheme 2ª



2.1 X = O, S

2.2

2.3

2.4

2.5

2.6

ªNE = *N*-terminal extein; CE = *C*-terminal extein; IN = intein

(**2.2**) that then undergoes a second intramolecular acyl transfer (**2.2** → **2.3**) and cleavage (**2.3** → **2.4**), producing a transient intermediate (**2.4**). This rearranges either by intramolecular *O*→*N* or *S*→*N* acyl transfer (**2.4** → **2.6**) to give the coupled product (**2.6**).

Proximity effects have also been exploited *in vitro*[19] for protein semisynthesis and, in this approach, two peptide segments derived from a protein (with or without subsequent modification) are associated (either covalently[19e] or non-covalently) in such a way that the reactive *C*-terminal α-carboxyl of one segment and *N*-terminal α-amino group of the other are close together, thus facilitating acyl transfer. Self-association can occur due to the propensity of large peptide fragments to form complexes approximating the native conformation of the original protein but, given that the degree of complementarity necessary for self-association is quite specific, this approach to segment coupling is unlikely to be generally useful. However, as an outcome of the intein/extein studies summarized in Scheme 2, a new type of *in vitro* semisynthesis is being developed which may prove to be more general.[20] In this approach, the essential *N*-terminal portion of a particular intein (having an extein at its *N*-terminus) and the essential *C*-terminal portion of the same intein (having an extein attached to its *C*-terminus) are allowed to associate. This association results in an active splicing system, which ultimately links the exteins, forming a new peptide and excising the intein-like complex. The generality of this approach would arise from the ability to attach various exteins to the truncated intein segments.

In terms of developing a generalized method for coupling peptide segments by taking advantage of intramolecular acyl transfer processes, the ligation-based strategy mentioned above is extremely important. This approach offers several advantages[21] over conventional techniques for peptide segment coupling, as these have a number of characteristics that cause problems when applied to the coupling of large peptides.[12e,22,23] Firstly, given the large size of the peptide segments, an entropic barrier exists that reduces coupling efficiency. This comes about because the terminal α-amino group of the amine component and the activated terminal acyl group of the acyl component do not have a high enough reactivity to make the intermolecular reaction sufficiently rapid at the high dilutions necessary to solubilize large peptide fragments; competing *intramolecular* side reactions then occur. Secondly, protection of the side-chain functional groups is necessary in order to prevent undesired reactions between the activated acyl group and peptide nucleophiles other than the intended amino group. This situation requires that a large number of protecting groups be removed in the final step of the synthesis — an operation which also leads to problems of product purification. Moreover, the required activation of the carboxyl terminus of the *N*-terminal peptide renders coupling of the segments susceptible to epimerization at the center adjacent to the activated acyl group.[7b] Finally, in order to prevent solvolysis of the activated terminal acyl group during the coupling process, dipolar aprotic solvents are required. Unfortunately, peptides are often poorly soluble in these solvents and tend to associate significantly in them — a factor that further inhibits the coupling process.

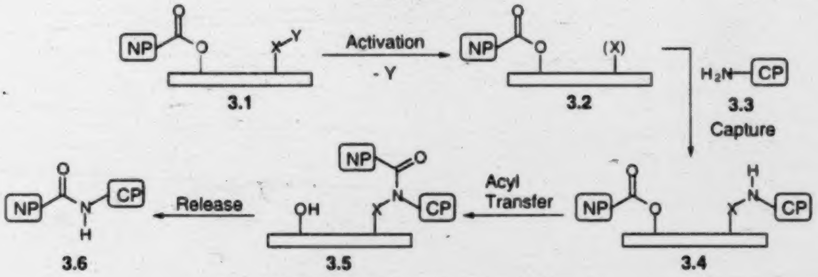
The advantages of a ligation-based approach can be outlined as follows. Since the relatively weak mutual reactivity of the terminal acyl carbon of one fragment and the terminal amino nitrogen of the other cannot be relied on to join the peptide chains, a separate and efficient ligation reaction is used to link the fragments and bring the two coupling sites into proximity. Once the fragments have been ligated, formation of the peptide bond will follow first order kinetics and, consequently, factors such as steric hindrance by a bulky α-substituent on either of the amino acid residues involved in the coupling should exert less significant retarding effects compared with corresponding intermolecular reactions. Additionally, placing the terminal amino and the terminal acyl groups close together results in a high local concentration of these species, and this imparts a strong entropic advantage to the system, thereby removing the need for enthalpic activation of the acyl group. Given that acyl transfer now involves a relatively unactivated acyl group, the likelihood of epimerization or reaction with nucleophiles other than the closely-positioned terminal amine is decreased and, consequently, the requirement for protecting groups is diminished or even removed. It may be possible, therefore, to use unprotected peptide segments in aqueous solution, and this approach would most likely avoid solubility problems, for example, and facilitate purification.

1.3 Prior Amine Capture Strategies

The first general approach to a ligation-based coupling technique is the *Prior Amine Capture Strategy* reported by Kemp in 1975.[21,24]

The principles of the *Prior Amine Capture Strategy* can be outlined in a general way by reference to Scheme 3. Structure **3.1** represents the *N*-terminal peptide chain that is ultimately to undergo coupling with the *C*-terminal chain, and is derivatized at its *C*-terminus in the form of a weakly activated ester. The alcohol portion of this ester is a template which, in the ligated product (*cf.* **3.4**), will serve to position the appropriate amino and acyl groups in proximity so that rapid intramolecular acyl transfer can occur. The group represented as X-Y in **3.1** is the protected form of one of the functional groups involved in the initial ligation and is referred to as the *capture site*. In this case, X is the capture atom and Y is its protecting group. The capture site is capable of bonding to the terminal α-amino group of the *C*-terminal peptide (**3.3**).

Scheme 3[a]



[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

The ligation is initiated by removal of the protecting group (**3.1** → **3.2**) so as to activate the capture site. Capture (**3.2** + **3.3** → **3.4**) then occurs in the presence of the *C*-terminal segment to give the system represented as **3.4**. At this point intramolecular acyl transfer takes place giving the rearranged system **3.5**. Finally, release of the template from **3.5** by cleavage of the X-N bond (**3.5** → **3.6**) results in formation of the coupled peptide **3.6**, which contains a new peptide bond linking what were originally two separate peptides.

In order to reduce the above plan to practice, Kemp recognized that several important criteria had to be met. First, a versatile, clean, and reliable method of attaching a template (containing a masked capture group) to the *C*-terminus of the *N*-terminal peptide (*cf.* **3.1**) would have to be found and, second, the template itself would have to meet certain criteria. For instance, the template would need to withstand all reaction conditions met during the coupling process but still be removable without damage to the newly formed peptide. The template must, of course, also correctly position the acyl and amino components close together so as to facilitate intramolecular acyl transfer.

Another prerequisite for implementing the *Prior Amine Capture Strategy* is that a suitable capture step be developed. This step would have the crucial role of bringing the peptide segments together before peptide bond formation; it would have to occur rapidly and efficiently, and also in a solvent that inhibits self-association of peptides — a phenomenon that can, for example, decrease solubility and/or restrict access to reaction sites.

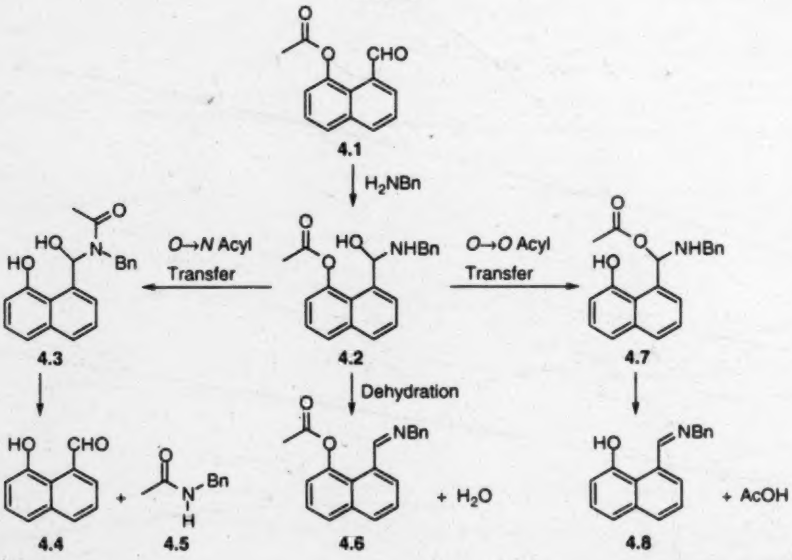
With the above objectives in mind, Kemp examined two general approaches for amine capture. In one, a carbonyl group is the electrophilic capture site (see 1.3.1 and 1.3.2) and, in the other, a nitro olefin is used (See 1.3.3).

1.3.1 Ligation by Hemiaminal Formation

Initial studies of the *Prior Amine Capture Strategy* involved examination of 8-

hydroxy-1-naphthaldehyde (**4.4**, Scheme 4) as a potential candidate both for promoting amine capture and for serving as a template to allow acyl transfer.[25] It was thought that initially a hemiaminal would be formed and that this species would act as an intermediate through which intramolecular acyl transfer could occur. In order to test the idea, aldehyde **4.1** was allowed to react with benzylamine in DMSO; *N*-benzylacetamide (**4.5**), the *O*→*N* acyl transfer product, was isolated in 70% yield, along with acetic acid (30% yield). This result suggested that the hemiaminal did indeed form (**4.1** → **4.2**), and that it then underwent both *O*→*N* acyl transfer (**4.2** → **4.3**) and *O*→*O* acyl transfer (**4.2** → **4.7**).

Scheme 4



The ratio of products was solvent dependent (Table 1), but no conditions were found that led exclusively to formation of **4.5**. In DMF the ratio of **4.5** to acetic acid was the same as in DMSO. When 1:1 DMSO-water was used, a product ratio of 3:7 was observed, and with 1:4 acetonitrile-water, the product ratio was 1:9. Competing imine formation (**4.2** → **4.6**) was significant in certain other solvents. For example, use of acetonitrile resulted in formation of **4.5** and **4.6** in a 1:1 ratio. In some instances no product corresponding to *O→N* acyl transfer was detected. This was the case when the reaction was carried out in benzene, carbon tetrachloride, or chloroform; **4.5** was not observed, whereas both acetic acid and **4.6** were, in ratios of 7:3, 7:3, and 1:4, respectively.

Table 1[25]

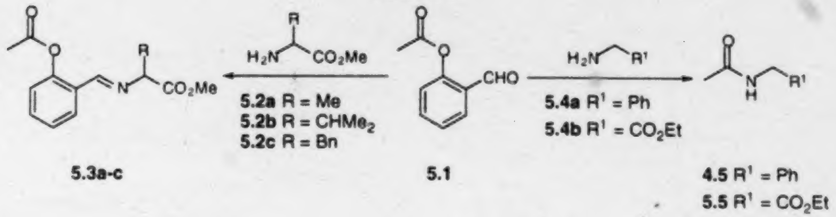
Effect of Solvent on the Product Distribution for Reaction of **4.1** with Benzylamine

	Relative Product Ratios		
Solvent	*O→N* Acyl Transfer Product (**4.5**)	*O→O* Acyl Transfer Product (AcOH)	Dehydration Product (**4.6**)
DMSO	7	3	0
DMF	7	3	0
DMSO-H_2O (1:1)	3	7	0
MeCN-H_2O (1:4)	1	9	0
MeCN	1	0	1
Benzene	0	7	3
CCl_4	0	7	3
$CHCl_3$	0	1	4

In this system (Scheme 4), the intramolecular acyl transfer proceeds through a seven-membered transition state, and rate constants for the combined *O→N* and *O→O* acyl transfer processes in acetonitrile, DMF and 1:4 acetonitrile-water were found to be 0.1, 0.2, and 15 $M^{-1}sec^{-1}$, respectively. An estimation of the rate of the *intermolecular* reaction between benzylamine and the acetyl group of **4.1** was made by measuring the rate constant for reaction between benzylamine and 8-acetoxy-1-nitronaphthalene. The value was 1 x 10^{-3} $M^{-1}sec^{-1}$ in acetonitrile. Since reaction of **4.1** with benzylamine (also in acetonitrile) had a rate constant two orders of magnitude greater (i.e. *ca.* 1 x 10^{-1} $M^{-1}sec^{-1}$), the intramolecularity of the acyl transfer via **4.2** was implied, although not unambiguously proven.

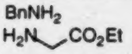
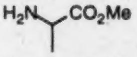
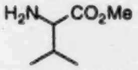
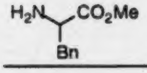
The hemiaminal approach to ligation was further explored[25] by examining two other templates. The first was 2-acetoxybenzaldehyde (**5.1**, Scheme 5) which, when treated with benzylamine in any of the above solvents, gave **4.5** in quantitative yield, with rate constants close to those observed for **4.1** in DMF, acetonitrile, and 1:4 acetonitrile-

Scheme 5



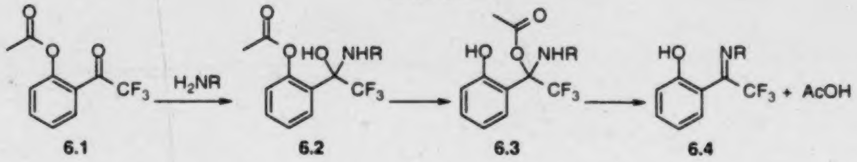
water. The assumption that formation of **4.5** was, in fact, the result of an intramolecular process was based on the observation that the corresponding reaction using the isomeric aldehyde 4-acetoxybenzaldehyde proceeded about 150 times slower. Although the reaction with **5.1** was very efficient, experiments with other amines revealed that the product composition depended on the amine used (Table 2). For example, reaction of ethyl glycinate (**5.4b**) with 2-acetoxybenzaldehyde in benzene resulted in a 90% yield of the *O*→*N* acyl transfer product (**5.5**). However, the methyl esters of alanine (**5.2a**), valine (**5.2b**), and phenylalanine (**5.2c**) each gave exclusively the imine derivatives (**5.3a-c**).[26]

Table 2[25]
Product Distribution when **5.1** Reacts with Different Amines

Amine	*O*→*N* Acyl Transfer Product (%)	Dehydration Product (%)
$BnNH_2$	100	0
	90	0
	0	100
	0	100
	0	100

2-Acetoxytrifluoroacetophenone (**6.1**) was also examined (Scheme 6) as a template for acyl transfer, in the hope that the intermediate hemiaminal (*cf.* **6.2**) would not be prone to dehydration.[25] When **6.1** was treated with any of the above-mentioned amines,

Scheme 6



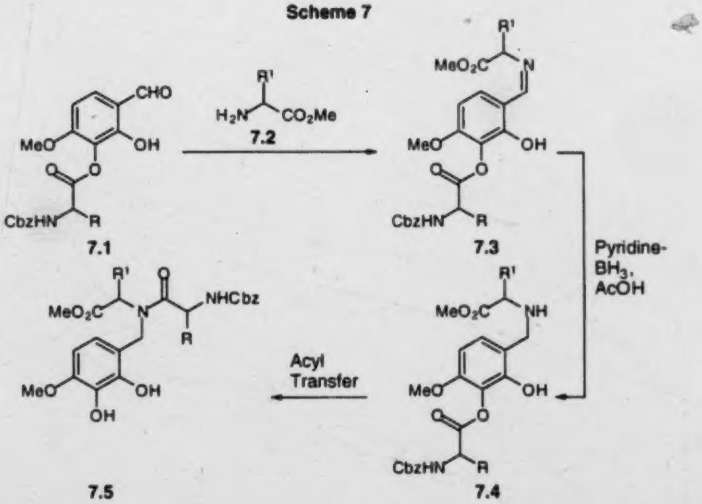
however, only products corresponding to *O→O* acyl migration (**6.1** → **6.2** → **6.3** → **6.4**) were observed and, in each case, acetic acid was isolated in quantitative yield. Evidently, direct dehydration of the intermediate hemiaminal was indeed avoided using **6.1** — but not with the intended result.

1.3.2 Ligation by Imine Formation

A slightly modified form of the hemiaminal approach — but still involving nucleophilic addition of an amine to a carbonyl group — was also examined.[27] This version avoids dehydration as an unwanted side reaction by actually incorporating the loss of water into the capture process.

The idea was tested using **7.1** (Scheme 7) as the amine capture device.[27] Here, an

Scheme 7



imine is formed (**7.1** + **7.2** → **7.3**) by dehydration of the hemiaminal generated from the amine and the aldehyde. Direct and facile formation of the imine was expected, as such behavior is characteristic of salicylaldehydes.[28] Both R and R^1 were varied (R = H, R^1 = Me, CH_2CHMe_2, Bn; R = Me, R^1 = Me; R = Bn, R^1 = $CHMe_2$) to give the corresponding imines; in all cases imine formation was efficient (**7.1** + **7.2** → **7.3**). The imines were then reduced (**7.3** → **7.4**), using pyridine-borane in acetic acid, to give a secondary amine that underwent intramolecular acyl transfer (**7.4** → **7.5**).[29] The transfer was slowed somewhat by the use of polar solvents such as DMSO, but proceeded readily in other solvents. When R = H, the half-times for acyl transfer to captured AlaOMe, LeuOMe, and PheOMe in deuterochloroform were 15, 40, and 70 min, respectively (Table 3). Variation of the acyl fragment also influenced the rate; with R = Me or Bn half-times to captured AlaOMe were 70 and 120 min, respectively. Yields for the acyl transfer reaction were good. For example, when **7.1** (R = H) was treated with (±)-PheOEt, the corresponding acyl transfer product (*cf.* **7.5**) was obtained in 85% yield. The method could also be used to make a tripeptide.[27] Thus, reaction of **8.1** (= **7.1**, R = H) with LeuGly tetramethylguanidine salt (**8.2**, Scheme 8), followed by imine reduction and acyl transfer, gave **8.3** in 92% yield.

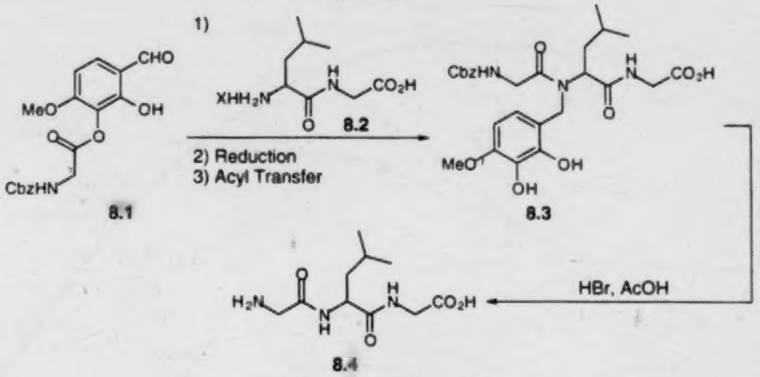
Table 3[27]

Half-Times for Intramolecular Acyl Transfer Reactions of 7.4

Acylating Component (7.1)	Amine Component (7.2)	Acyl Transfer Half-Time (min)
R = H	R^1 = Bn	70
H	CH_2CHMe_2	40
H	Me	15
Me	Me	70
Bn	Me	120

Cleavage of the 4-methoxy-2,3-dihydroxybenzyl moiety from the rearranged products was easily and efficiently achieved by treatment either with HBr-AcOH or with TFA in the presence of resorcinol.[27] For example, cleavage from the tripeptide derivative

Scheme 8[a]



[a]XH = tetramethylguanidine

8.3, with concomitant removal of the amine protecting group, by HBr-AcOH, gave GlyLeuGly (**8.4**) in 84% yield.

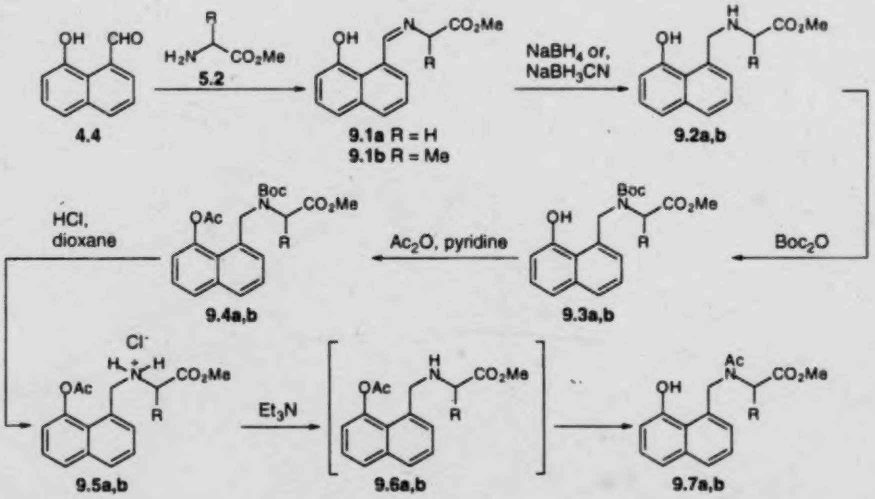
Given that the acyl group in the procedures of Schemes 7 and 8 is only weakly activated as an *O*-aryl ester, epimerization at the adjacent stereogenic center was unlikely.[30] However, the stereochemical integrity with respect to the α-carbon of the captured amino acid, once it had been derivatized as an imine, was of concern, and so the extent, if any, of epimerization at this center was examined, using an isotopic dilution assay.[30] When **8.1**, which was ^{14}C-labeled at the glycine α-carbon, and L-PheGlyOEt were allowed to react in acetonitrile for periods of 1.5 and 14 h, the *racemic* tripeptide was obtained in 0.1% and 0.3% yield, respectively, after the complete sequence of imine formation, imine reduction, intramolecular acyl transfer, and cleavage with TFA. This result indicated that the extent of epimerization was very low.

The approach of Scheme 7 is a practical example of peptide bond formation using a ligation-based technique. However, although the amine capture step worked well, the low rates of acyl transfer showed that refinement of the template was still required.

An efficient intramolecular acyl transfer is one in which a clean and rapid reaction that is not overly sensitive to steric factors is observed[31] and, in an effort to better satisfy these criteria, two other templates (see later, Schemes 9 and 10) were examined as candidates for facilitating acyl transfer. These templates differ from one another in the size and shape of the cyclic transition state through which the acyl transfer must occur.

Compounds **9.6a,b** (Scheme 9), derived from 8-formyl-1-naphthol, provided a second opportunity to examine acyl transfer through a seven-membered transition state.[32] The corresponding hydrochloride salts (**9.5a,b**) were derived from 8-formyl-1-naphthol (**4.4**) as follows. Treatment of the naphthol with either GlyOMe or AlaOMe gave an imine (**4.4** → **9.1a,b**), which was subsequently reduced and protected as its *N*-Boc derivative (**9.1a,b** → **9.2a,b** → **9.3a,b**). The phenolic hydroxyl was acylated with acetic anhydride in the presence of pyridine, and deprotection of the secondary amine, using HCl in dioxane, gave the hydrochloride salts of **9.6a,b** (**9.3a,b** → **9.4a,b** → **9.5a,b**). Treatment of each salt with triethylamine served to generate **9.6a,b** *in situ*, and the liberated amines underwent acyl transfer, giving amides **9.7a,b** (**9.5a,b** → **9.6a,b** → **9.7a,b**).

Scheme 9



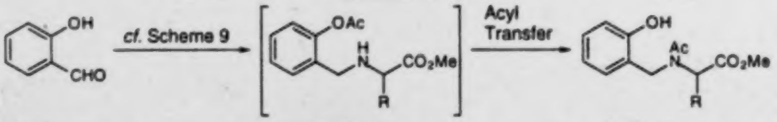
The acyl transfer reactions of **9.6a,b** were studied in a variety of solvents. Half-times for the glycine derivative **9.6a** in acetonitrile, chloroform, DMF, and DMSO were 3.6, 9, 50, and 36 min, respectively (Table 4), but a much slower reaction was observed with the more sterically hindered alanine derivative **9.6b**, which exhibited half-times of 3.3 and 3.5 h in acetonitrile and chloroform, respectively. Hence, the rate of acyl transfer via a seven-membered transition state with the naphthalene template is sensitive to steric effects, as shown by the Ala/Gly half-time ratios of 55 and 23 in acetonitrile and chloroform, respectively.

Table 4[32]
Half-Times for Intramolecular
Acyl Transfer Reactions of **9.6a,b**

Acyl Transfer Substrate	Solvent	Acyl Transfer Half-Time
9.6a	MeCN	3.6 min
	$CHCl_3$	9 min
	DMF	50 min
	DMSO	36 min
9.6b	MeCN	3.3 h
	$CHCl_3$	3.5 h

The related acyl transfer via a six-membered transition state was also examined further by studying **10.2a-c** (Scheme 10),[32] obtained from 2-hydroxybenzaldehyde (**10.1**) in a manner analogous to the formation of **9.6a,b**. The rates of acyl transfer for **10.2a-c** were measured in several dipolar aprotic solvents and, for **10.2b** and **10.2c**, also in water (pH 10) (Table 5). In general, the acyl transfer reactions proceeded most readily in water, having half-times of 24 sec for **10.2b** and 3.3 min for **10.2c**. When aprotic dipolar solvents were used, the highest rate of acyl transfer for each of the

Scheme 10



10.1

10.2a R = H
10.2b R = Me
10.2c R = $CHMe_2$

10.3a-c

compounds was observed in acetonitrile, where half-times of 36 sec, 50 min, and 4.5 h were measured for **10.2a-c**, respectively. Acyl transfer was slowest in DMF in the case of **10.2a** and **10.2b** [acyl transfer for **10.2c** was measured only in water (pH 10) and acetonitrile]. In comparison to the seven-membered acyl transfer systems **9.6a**,**b**, the present templates (**10.2a-c**) exhibited consistently greater acyl transfer rates and, with the exception of the reaction in chloroform, where the rate for **10.2a** was only 1.3 times as fast as for **9.6a**, the rate of acyl transfer for **10.2a** was on average 5.7 times as fast as that for **9.6a** in the solvents examined. The alanine derivatives also showed a similar trend, with **10.2b** reacting 1.6 times as fast as **9.6b** in chloroform, and 4.0 times as fast in acetonitrile. Like its seven-membered counterpart, the acyl transfer system based on **10.1** was sensitive to the steric bulk of the captured amine, and the effect was even more pronounced in this case. In acetonitrile, the Ala/Gly half-time ratio was 83 and the Val/Gly half-time ratio was 450.

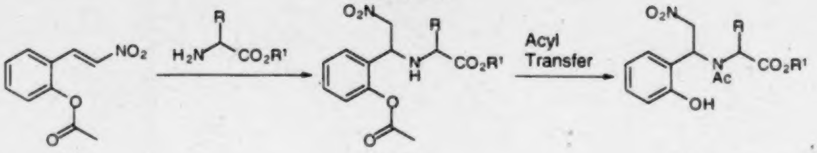
Table 5[32]
Half-Times for Intramolecular
Acyl Transfer Reactions of **10.2a-c**

Acyl Transfer Substrate	Solvent	Acyl Transfer Half-Time
10.2a	MeCN	36 s
	DMSO	6 min
	$CHCl_3$	7 min
	DMF	10 min
10.2b	H_2O (pH 10)	24 s
	MeCN	50 min
	$CHCl_3$	2.2 h
	DMSO	11.5 h
	DMF	12.5 h
10.2c	H_2O (pH 10)	3.3 min
	MeCN	4.5 h

1.3.3 Ligation by Michael Addition

As an alternative to the carbonyl-containing templates described above (Sections 3.1 and 3.2), the nitrostyrene derivative **11.1** (Scheme 11) was evaluated for its ability to act as an amine capture device, and also as a template to mediate intramolecular acyl transfer.[31]

Scheme 11



11.1 **11.2** **11.3**

Amine capture in this case occurs by Michael addition of the primary amine function to the nitro olefin (**11.1** → **11.2**) and positions the acyl group and the amine nitrogen in such a way that intramolecular acyl transfer (**11.2** → **11.3**) can occur. It was expected that this amine capture system should have certain inherent advantages compared to the templates discussed above. First, unlike approaches involving a hemiaminal intermediate, problems of competing elimination or *O*→*O* acyl transfer cannot arise. The required Michael addition was also expected to be efficient, as reaction of β-nitrostyrene with primary amines is known to proceed rapidly and in nearly quantitative yield.[33] Secondly, the capture reaction gives a secondary amine directly, thus avoiding the reduction step required in systems where capture results in an imine.

Michael addition between **11.1** and the amino acid esters GlyOEt, AlaOMe, PheOMe, and ValOMe was easily carried out in acetonitrile, and the adducts were then tested for their ability to undergo acyl transfer. Despite the fact that the required intramolecular transfers were occurring through a presumably favorable six-membered transition state, slow reactions were observed in all cases, and the rate constants are listed in Table 6. The data reveal a Gly/Ala rate ratio of 100, a Gly/Phe rate ratio of 286, and a

Table 6[31]

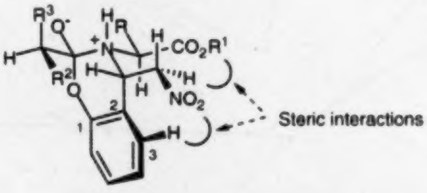
Rates of Acyl Transfer in **11.2**

Acyl Transfer Substrate (**11.2**)	Acyl Transfer Rate (min^{-1})
R = H, R^1 = Et	0.02
R = Me, R^1 = Me	2×10^{-4}
R = Bn, R^1 = Me	7×10^{-5}
R = $CHMe_2$, R^1 = Me	2×10^{-5}

Gly/Val rate ratio of 1000, suggesting a strong dependency of the rate on steric factors. Replacement of the acyl fragment (MeCO) with $CbzHNCH_2CO$ showed no significant changes in the rate of acyl transfer.

The steric effects observed in this study were rationalized by analogy to a previously established model that dealt with steric effects on aminolysis of peptide *p*-nitrophenyl esters.[34] Based on this model, the structure of the transition state leading to acyl transfer in compounds **11.2** was proposed to be **12.1** (Scheme 12), or a distereomer. From the diagram, it can be seen that steric interactions exist between the nitromethylene group (CH_2NO_2) and either the ester (CO_2R^1) or α-alkyl substituent (R) of the amino acid, as well as between the nitromethylene group and H(3) of the aromatic template. Structure **12.1** is not capable of undergoing any stabilizing conformational changes that relieve these interactions without introducing new ones.

Scheme 12[a]



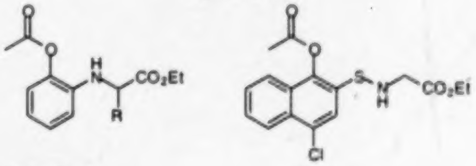
12.1

[a] R^2 = *N*-terminal peptide; R^1 = *C*-terminal peptide; R, R^3 = amino acid side-chains

The above model suggests that sensitivity to steric factors should be expected in any derivative of **3.4** (Scheme 3) except where the capture site X is small and, especially, if it has no substituents (*cf.* the CH_2NO_2 group of **12.1**). For instance, minimal steric factors would be expected if X is sulfur, a methylene group, or an sp^2 atom. Consideration of model **12.1** seemed to emphasize the fact that unfavorable steric interactions can nullify entropically favorable ring sizes for the transition state. However, a way to avoid such steric interactions is to modify the link — and its attachments — that join the captured amine to the template.

The case where the link is a simple methylene group had been dealt with to some extent by examination of systems **7.4**, **9.6**, and **10.2** (see above), and an attempt[31] was then made to test the effect of having X as an sp^2 atom. This was done[31] by examining acyl transfer rates for the ethyl esters of *N*-(2-acetoxyphenyl)glycine (**13.1a**), *N*-(2-acetoxyphenyl)alanine (**13.1b**), and *N*-(2-acetoxyphenyl)valine (**13.1c**). Intramolecular *O*→*N* acyl transfer, which proceeded through a five-membered transition state in these compounds, was very slow. The poor nucleophilicity of the nitrogen atom (it is part of an aniline system), and the development of strain in the transition state, were regarded as factors responsible for the low rates. The Gly/Ala and Gly/Val rate ratios were comparable to those observed in corresponding intermolecular reactions.

Scheme 13



13.1a R = H
13.1b R = Me
13.1c R = CHMe₂

13.2

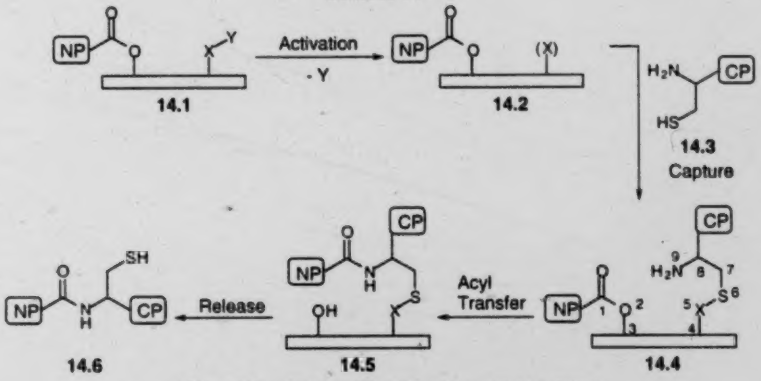
Finally, the effect of using a sulfur atom as the link was studied briefly[31] by examination of **13.2**, but even for the unhindered case shown, the rate of intramolecular acyl transfer was very low (3×10^{-4} min^{-1}) — for reasons that were not identified.

1.4 Prior Thiol Capture Strategies

As a result of the investigations described above, it had become apparent that a coupling strategy in which acyl transfer proceeds through a relatively small transition state of five to seven members is not effective, due to steric crowding. The suspicion had also developed that the amino group might not be nucleophilic enough to achieve rapid and clean capture at high dilution. On the matter of steric crowding, it appeared that in the ligation product the segment linking the amino and acyl components was either too inflexible, or failed to position them properly. Consequently, there were limited opportunities for avoiding unfavorable nonbonded interactions in reaching a suitable transition state for acyl transfer. Kemp reasoned that the best way to avoid these unfavorable interactions was simply to increase the length of the spacer arm while maintaining its rigidity. The problem of low nucleophilicity of the amine nitrogen, on the other hand, had to be dealt with by finding an entirely new way of ligating the peptide segments.

An extremely elegant approach to these apparently separate problems was developed[35] through a single modification of the initial strategy. The modification required that cysteine be the *N*-terminal residue of the *C*-terminal peptide chain. Of course, this imposes the limitation that the coupling site between the two peptides has to be between that cysteine and another amino acid. The approach, which is referred to as the ***Prior Thiol Capture Strategy***, is outlined in Scheme 14.[35] In the first step, the masked capture site (X-Y) of the template is activated (**14.1 → 14.2**); it then undergoes reaction with the thiol function of the *N*-terminal cysteine to give the ligated product (**14.2 + 14.3 → 14.4**).

Scheme 14[a]



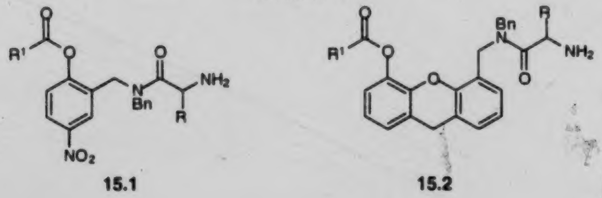
[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

Intramolecular acyl transfer (**14.4** → **14.5**) ensues, forming the rearranged system **14.5**, which contains a new peptide bond. Finally, cleavage of the template (**14.5** → **14.6**) liberates the newly-formed peptide **14.6**.

The potential of this strategy to overcome the limitations of the *Prior Amine Capture Strategy* can be explained as follows. The thiol function of the terminal cysteine is strongly nucleophilic and, in contrast to the amino group that is involved in the *Prior Amine Capture Strategy*, this difference should greatly increase the likelihood of effecting smooth ligation of the peptide segments prior to acyl transfer, even at high dilution. Moreover, the ensuing acyl transfer transition state would necessarily consist of at least nine atoms (*cf.* **14.4**) and, therefore, could provide ample conformational freedom to overcome unfavorable interactions resulting from steric crowding. Before trying the *Prior Thiol Capture Strategy*, however, two matters required attention. The first was to determine what type of ligation process would best take advantage of the reactivity of the thiol group, and the second was to establish if acyl transfer could proceed through a transition state as large as that necessitated by this approach.

The consequences of a larger acyl transfer transition state had already been evaluated to some extent during earlier work[32] in which intramolecular acyl transfer in **15.1** and **15.2** (Scheme 15) had been examined. The transfer in these systems proceeds via transition states of nine and twelve members, respectively. In each system, R and R^1 were varied extensively, and the rates of acyl transfer were measured in a variety of solvents. These studies showed that acyl transfer could, indeed, proceed efficiently

Scheme 15



15.1 **15.2**

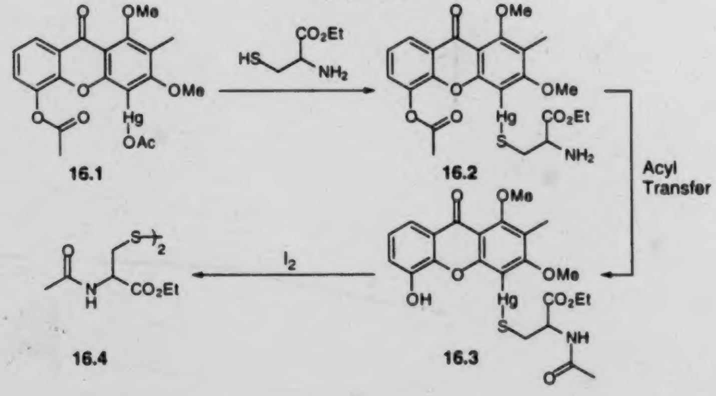
through transition states involving medium to large rings, as indicated by Scheme 14.

The question of what type of ligation process would best take advantage of the reactivity of the thiol group was examined by studying both mercaptide formation (Section 4.1) and disulfide formation (Section 4.2).

1.4.1 Ligation by Mercaptide Formation

Kemp's early work on ligation by thiol capture involved the use of an organomercury derivative.[36] Compounds of this type were chosen because they have a high affinity for thiol groups and react rapidly with them.[37] The C-Hg-S linkage that would be produced by ligation has a linear geometry and, in order to test acyl transfer via the resulting large ring that is necessarily formed, compound **16.2** was studied. This compound was generated *in situ* (Scheme 16) by treating **16.1** with the ethyl ester of cysteine at concentrations ranging from 10^{-2} M to 10^{-3} M in either DMF or DMSO. Half-times for the acyl transfer (**16.2** → **16.3**) were found to be 24 and 8 h in DMF and DMSO, respectively, and were independent of concentration, suggesting that transfer was intramolecular. The template was detached from the rearranged product by iodine-mediated oxidation (**16.3** → **16.4**), so as to release the disulfide **16.4**.

Scheme 16



1.4.2 Ligation by Disulfide Exchange

Capture by disulfide formation was also examined[38,39] as a means of ligating two peptide segments prior to their coupling by acyl transfer. In this approach, which has also been explored in a modified form (see later, Scheme 66), (X) in structure **14.2** (Scheme 14) takes the form of a thiol group, and ligation occurs by disulfide formation between this template thiol and the cysteine thiol at the *N*-terminus of the *C*-terminal peptide. Intramolecular acyl transfer from **14.4** (X = S) generates the peptide bond, and then cleavage of the resulting disulfide **14.5** (X = S) liberates the newly formed peptide **14.6**. Several practical points had to be considered in order to develop this version of the *Prior Thiol Capture Strategy*, and Kemp has carried out extensive research on each of these points.

(a) Template Design

Implementation of the *Prior Thiol Capture Strategy* requires a template that allows rapid intramolecular acyl transfer of a weakly activated acyl function to the weakly nucleophilic amino group of a cysteine residue.[38,40] As indicated in Scheme 14, the transfer necessarily involves a ring of at least nine atoms (*cf.* **14.4**, X = S). In general, cyclization of medium rings is entropically unfavorable.[13c,41] For this reason, the template would have to be constructed in such a way that, not withstanding the ring size of the transition state, the amine and the acyl group are easily positioned in a manner that confers a proximity-based entropic advantage, thus making the lack of enthalpic activation at the acyl substituent irrelevant.

(i) Effective Molarity

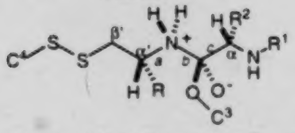
The entropic advantage for an intramolecular reaction, which results as a consequence of restraining the nucleophilic and the electrophilic components in proximity, can be expressed quantitatively in terms of *effective molarity* (EM).[13b,c] The EM for a system can be estimated when a corresponding intermolecular version of a particular reaction exists. In such a case, the EM of the system is expressed as the ratio of the first order rate constant (for the intramolecular reaction) to the second order rate constant (for the intermolecular one). In practice, EM is equal to the concentration of the external electrophile or nucleophile that must be added to the intramolecular reaction mixture so that both intra- and intermolecular reactions proceed at the same rate.

Kemp has used the concept of EM to guide the development of an optimal template for acyl transfer.[40,42] This was done by measuring the EM with various templates that were deemed to possess the potential to function in the required manner, and then excluding from further study those with an unacceptably low EM value, the minimum value being set at EM = 1 M. An effective template would be associated with both a high EM and a high rate of intramolecular acyl transfer.

(ii) Transition State Model

In order to aid the design of suitable templates for the disulfide approach, a transition state model was proposed[32,40] on the basis of the following considerations. Information from earlier studies[43] justified the assumption that the transition state has nearly tetrahedral geometry at both the acyl carbon and the amine nitrogen, a fully formed C-N bond, and partially broken N-H and C-O bonds (Scheme 17). Furthermore, by analogy to a previously reported transition state model for intermolecular aminolysis of *p*-nitrophenyl esters,[34] it was assumed for the present purposes that the bond which connects the α-carbon (see Scheme 17) and the acyl substituent (bond *c*), and the bond which connects the α'-carbon and the amino substituent (bond *a*) have an antiperiplanar relationship about the C-N bond (bond *b*) in the acyl transfer transition state. Further refinement[40] of the transition state model was carried out by applying assumptions regarding bond angles and the conformational preferences of the bonds not already defined. The result of these considerations was the transition state model **18.1** (Scheme 18).[40]

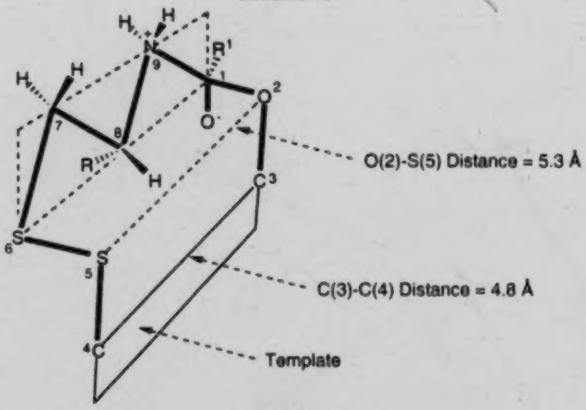
Scheme 17[a]



[a]R¹ = *N*-terminal peptide; R = *C*-terminal peptide; R² = amino acid side-chain

This structure implies that an ideal template would be one that can accommodate the particular geometric arrangement of atoms shown and provide a framework connecting C(4) and C(3) by a distance of 4.8 Å, and do so in a way that the resulting C(4)-S(5) and C(3)-O(2) bonds are oriented to give an O(2)-S(5) distance of 5.3 Å. It was also deemed important that the template be rigid, so as to decrease the number of random conformations that the system could sample before reaching the required transition state conformation.

Scheme 18[a]



18.1

[a]R¹ = *N*-terminal peptide; R = *C*-terminal peptide

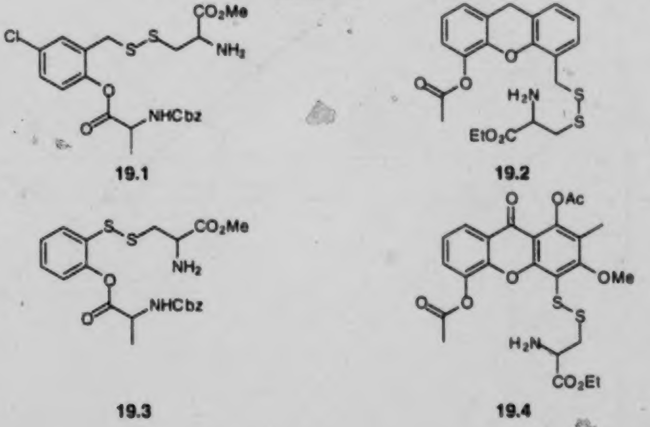
Finally, in order to avoid any major van der Waals interactions with the α-hydrogen [i.e. with H(8)] of the cysteine residue, the template should be free of substituents that protrude towards H(8); planar structures satisfy this requirement to some extent. Given that the model shown in Scheme 17 has rotational degrees of freedom in the C(α')-C(β')-S-S region, other low energy conformations must be accessible and, indeed, as indicated below, subsequent experiments indicated that **18.1** is not the only model that corresponds to a low-energy transition state for acyl transfer, but it was nevertheless adopted as the working model.

(iii) Evaluation of Templates

While model **18.1** emerged from the theoretical considerations given above, Kemp also included related structures as candidates for evaluation, thus broadening the survey and providing an opportunity to identify the effects of template flexibility and transition state conformation.

The first set of template-bound amino acids corresponding to model **18.1** that were examined are those shown in Scheme 19.[35] When dissolved in DMSO[44] for 20-40 h, compounds **19.1** and **19.2** each gave products corresponding to acyl transfer. However, the rates of transfer were low, and products resulting from disulfide exchange were also detected. Notwithstanding these undesirable features, each compound displayed a high EM. An accurate value could not be obtained due to the formation of byproducts, but an EM in the range of 3-14 M was estimated for **19.2**,[42,45] and 0.5-10 M[42] for **19.1**. These

Scheme 19

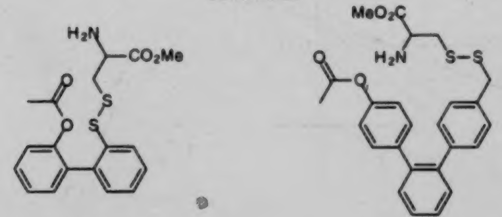


compounds have C(3)-C(4) distances (*cf.* Scheme 18) of 2.53 and 4.16 Å, and O(2)-S(5) distances of 2.5-4.2 and 2.6-5.4 Å, respectively,[42] and so only **19.2** approaches the values specified by the transition state model, but neither of them provides a sufficiently rigid framework, as indicated by the range of O(2)-S(5) distances that they can accommodate.

Compounds **19.3** and **19.4** were found[35] to undergo concentration-independent acyl transfer in both DMSO and DMF. The half-time for reaction of **19.4** in DMSO was 2.7 h,[35] and in DMF it was *ca.* 28 h.[35] An estimate of the EM of **19.4** was made[35] by determining the rate of intermolecular aminolysis for the reaction between ethyl *S*-benzyl cysteine and 1,3-dimethoxy-2-methyl-5-acetoxyxanthone. From this experiment, an EM of 0.5-0.7 M was determined[35,42] for the system. For **19.3**, an EM value between 0.08 and 1.3 M was estimated.[42] The C(3)-C(4) distances for compounds **19.3** and **19.4** were 1.40 and 4.76 Å, respectively, and the O(2)-S(5) distances were 2.98 and 4.81 Å, respectively.[42] Thus, although each of these compounds provided adequate rigidity, only **19.4** provided distances required of the transition state model **18.1**.

Compounds **20.1** and **20.2** were next examined for their ability to facilitate intramolecular acyl transfer (Scheme 20).[42,46] In the case of **20.1**, both the C(3)-C(4) and the O(2)-S(5) distances fell short of the optimal values, and for **20.2**, both were larger than desired.[42] In addition, each compound showed a range of values for both distances and lacked the required rigidity. When either **20.1** or **20.2** was kept in DMF, no products

Scheme 20

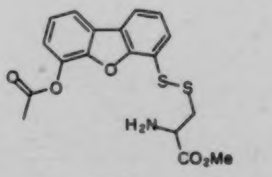


20.1 **20.2**

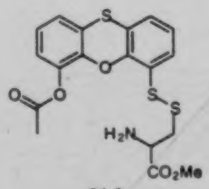
corresponding to acyl transfer could be detected, even after five days; only products of disulfide exchange were observed.[46] This result with **20.2** is noteworthy in the light of later work[42] which suggested that acyl transfer could also proceed through a more extended transition state than the one shown by **18.1**; such a transition state should be accessible to **20.2**, yet acyl transfer did not occur — presumably because of the flexibility of the system.[46]

The dibenzofuran **21.1** (Scheme 21) provided a promising example of a potential template for the *Prior Thiol Capture Strategy*, having a C(3)-C(4) distance of 4.82 Å and an O(2)-S(5) distance of 5.45 Å.[42] This template was examined along with the phenoxythiin system **21.2**,[40,42] which exhibited corresponding distances of 4.35 Å and 3.90 Å, respectively.[42] Efficient acyl transfer was not observed in the case of **21.2**,[40] and an EM of less than 0.1 M was determined.[40,42] Lack of acyl transfer in this system was attributed primarily to its inability to achieve a suitable O(2)-S(5) distance and, to some extent, to the flexibility of the system.[40,42]

Scheme 21



21.1



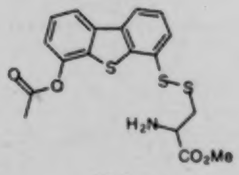
21.2

As was evident by the C(3)-C(4) and O(2)-S(5) distances given above, the dibenzofuran template (**21.1**) very effectively accommodated the structural requirements of the proposed transition state. The half-times for acyl transfer in this system were found[40] to be solvent dependent, with values of 23, 23, 2, and 2 h being observed in DMF, 5:1 hexafluoroisopropyl alcohol (HFIP)-DMSO, DMSO, and 1:5 DMSO-HMPA, respectively. In acetonitrile acyl transfer was not detected, even after 30 h. The half-time for the corresponding intermolecular reaction in DMSO was obtained by treating 4-acetoxydibenzofuran with ethyl *S*-benzyl-L-cysteinate. From this experiment, the EM of compound **21.1** was determined to be 4.6 M.

An examination of the effect of substituents attached to the phenolic ring of the dibenzofuran template on the rate of acyl transfer was undertaken.[40] As expected, an electron withdrawing substituent *para* to the phenolic oxygen caused a rate increase; in the case of a nitro group, for example, an increase of more than 3000 was observed.[40]

The sulfur analog **22.1** (Scheme 22) presumably has a degree of structural rigidity comparable to the dibenzofuran system, but the critical dimensions and the hypothesized transannular steric interactions with the cysteine α-hydrogen are quite different.[42] The C(3)-C(4) distance in **22.1** is 5.23 Å, whereas the O(2)-S(5) distance is 6.30 Å. This system did not undergo efficient acyl transfer[42] — a fact which lends support to the belief that the spatial relationships and nonbonded interactions implied by the transition state model **18.1** are important.

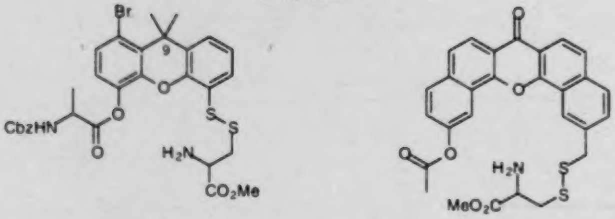
Scheme 22



22.1

The next set of templates studied included those shown in Scheme 23. The 9,9-dimethylxanthene derivative **23.1**[47] is structurally related to compound **19.2**, but possesses methyl substituents at C(9), which enforce a folded conformation on the ring system. The bromo substituent *para* to the acyl group was necessary because the reactivity of the unsubstituted system was too low to permit accurate monitoring of the intra- and intermolecular reactions. The EM calculated for this system was approximately 0.1 M,

Scheme 23



23.1

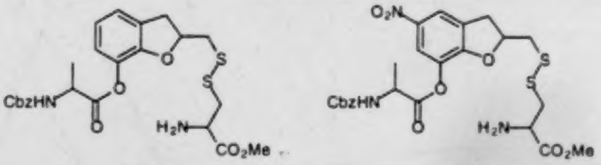
23.2

indicating that intramolecular acyl transfer, if it occurred at all, was not facilitated relative to the corresponding intermolecular process.

Compound **23.2**[47] provided an opportunity to test the assumption mentioned earlier in connection with compound **20.2** — that the acyl transfer could occur through a transition state with a more extended conformation than shown by model **18.1**. In the case of **20.2**, the absence of acyl transfer was attributed, in part, to its lack of rigidity.[46] Hence, studies with **23.2** would identify what benefit a rigid system might provide. In the event, the rate constant for the intramolecular process in DMSO was 0.02 h^{-1}, which corresponds to an EM of 0.3 M for **23.2**. Although the EM is low, the fact that acyl transfer occurred was regarded[47] as significant in that it further supported[48] the hypothesis that acyl transfer can take place through a more extended transition state, different from the one predicted by the model **18.1**.

Compound **24.1** and its nitro analog **24.2** were also evaluated.[47] These are fundamentally different from the previously studied systems, in that they provided an opportunity to examine intramolecular acyl transfer using a template containing a stereogenic center. The compounds embody the distances of Scheme 18 and the spanning atomic linkages of the dibenzofuran **21.1**, but their nonplanar conformations potentially reduce transition state crowding at the cysteine α-hydrogen. Both **24.1** and **24.2** were obtained as 1:1 mixtures of diastereomers, and the acyl transfer reaction of each diastereomeric mixture was examined.[47] The two diastereomers within each mixture reacted at different rates, and the rate constant for the more reactive isomer in **24.1** and **24.2** was found to be 0.013, and 2.3 h^{-1}, respectively. The rates of the corresponding intermolecular reactions were determined; the respective EMs for **24.1** and **24.2** (faster diastereomer in each case) were calculated as 6 M and 1.3 M.

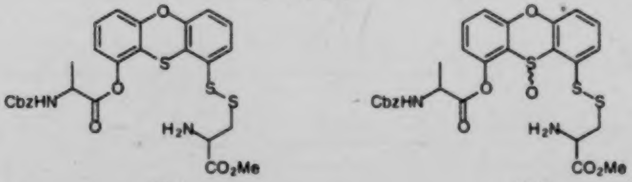
Scheme 24



24.1 **24.2**

Another report,[49] published at the same time, described the examination of compound **25.1**. It showed relatively low rates of transfer, with a corresponding EM of 0.3 M. Conversion of **25.1** into its sulfoxide derivatives **25.2** not only provided another opportunity to examine intramolecular acyl transfer across a template possessing a stereogenic center, but also offered the possibility of exploring what effect oxidation at the sulfur atom of **25.1** would have on the rate of reaction, since the presence of the sulfoxide group might facilitate acyl transfer in a manner similar to that of DMSO.[49] As expected, each of the (separated) diastereomers of **25.2** underwent acyl transfer at a different rate, and the higher rate constant was 7.0 h^{-1}. This corresponds to an EM of 0.22 M. Thus, compound **25.2** appears to be even less efficient as an acyl transfer system than **25.1**. The slower-reacting diastereomer of **25.2** had an EM of *ca.* 0.0015 M.

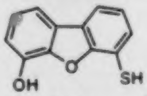
Scheme 25



25.1 **25.2**

During the course of the above research it had become apparent that 4-hydroxy-6-mercaptodibenzofuran (**26.1**, Scheme 26) was the best template of those examined, and was worthy of further study. In this template the C(3)-C(4) distance was found to be 4.82 Å, which compares well to the distance (4.8 Å) required by the transition state model **18.1**. The O(2)-S(5) distance of 5.45 Å was also close to the value of 5.3 Å specified by the model. In addition, the template provided the rigid framework that was needed, and was expected to show only one moderately weak van der Waals interaction between the furan oxygen and the cysteine α-hydrogen in the acyl transfer transition state.

Scheme 26



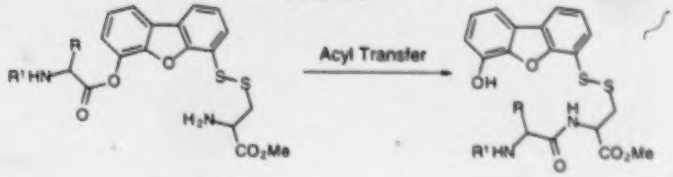
26.1

(b) Effect of Amino Acid Side-Chains on the Rate of Intramolecular Acyl Transfer

Since compound **26.1** emerged as a promising template for acyl transfer by the *Prior Thiol Capture Strategy*, Kemp undertook an investigation into the effect that the side-chain of the acylating component would have on transfer rates in this system.[50] It has been argued[34,50] that, for unassociated peptide segments, the rate of peptide bond formation is largely determined by the two substituents that neighbor the new bond. On this basis, it was recognized that coupling reactions giving dipeptides by intramolecular acyl transfer across the dibenzofuran template (*cf.* **27.1** → **27.2**, Scheme 27) should serve as suitable models for corresponding reactions in which actual peptide segments are coupled.

The rates of acyl transfer for the formation of several dipeptides using system **27.1** were measured in DMSO (Table 7)[50] and, with four exceptions [L-Pro, L-Val, L-Asp, L-Asp(*t*-Bu)], the transfer half-times were found to be between 2 and 4 h. The low rate of transfer in the case of the valine derivative was assumed to result from β-branching of the

Scheme 27

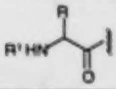
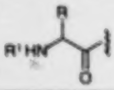


27.1 **27.2**

side-chain, a situation that is known to slow acyl transfer in intermolecular reactions as well.[34,51] In the case of the aspartic acid derivative, the high rate of transfer was rationalized on the basis of a stabilizing intramolecular hydrogen-bonding interaction (in the transition state) between the side-chain carboxyl group and the attacking amino group; this interpretation is consistent with the concept that an ideal template may well incorporate a hydrogen-bonding site.[50] The retarding effect associated with the proline residue was attributed to steric interactions in the transition state, but was deemed to be unique to that residue. Thus, intramolecular acyl transfer in **27.1** was tolerant of a range of substituent changes, but was significantly retarded by extreme steric interactions.

Table 7[50]

Half-Times for Intramolecular Acyl Transfer Reactions of **27.1**

	Acyl Transfer Half-Time (h)		Acyl Transfer Half-Time (h)
L-AlaGly	2.0	L-Asn	3.1
L-Ala	3.0	L-Asn(Mbh)	2.4
L-Leu	4.0	L-Asp(*t*-Bu)	1.1
L-Pro	34	L-Asp	~0.1
L-Val	51	L-Arg(Ans)	1.8
L-Lys(Cbz)	3.2	L-Thr(*t*-Bu)	2.5

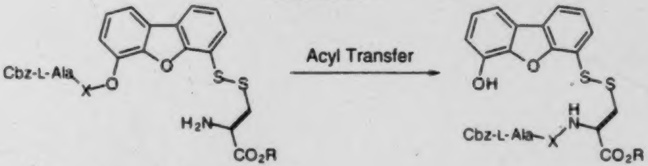
Mbh = 3,3'-dimethoxybenzhydryl; Ans = 9-anthracenesulfonyl

(c) *Extent of Epimerization*

The possibility of epimerization at the α-carbon of the acyl fragment during acyl transfer, using the dibenzofuran template **26.1**, was examined with the model systems

28.1a,b.[52] Given that the acylating component in these systems is only weakly activated as an aromatic ester, the epimerization levels were expected to be low, and this was indeed the case. Compound **28.1a** was allowed to react (Scheme 28) in DMSO for three days, in order to ensure complete acyl transfer, giving **28.2a**. This was then hydrolyzed in the presence of 12 M HCl-propionic acid to liberate the individual amino acid components. The L-isoleucine/D-*allo*-isoleucine ratio was measured and from this the extent of epimerization was determined to be 0.20 ± 0.27%, thus indicating that, within the limits of accuracy of the method, no epimerization occurs during the coupling process.

Scheme 28[a]



28.1a X = L-Ile, R = Me
28.1b X = L-Phe, R = Maq

28.2a,b

[a]Maq = 2-oxymethylanthraquinone

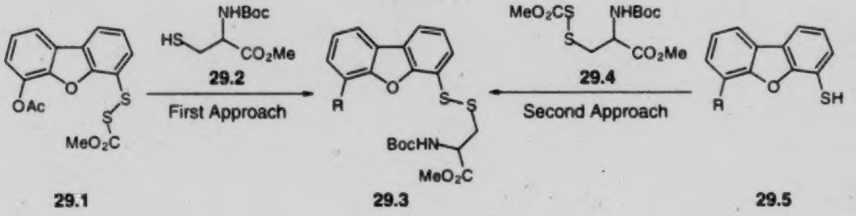
Further support for the absence of epimerization was obtained[52] by examining the acyl transfer products from **28.1b**. It had been established that the L-L-L form of **28.2b** was separable from its L-D-L epimer using HPLC, and that the separation could be achieved with baseline resolution. Hence, once the acyl transfer reaction for **28.1b** was complete, the products were analyzed using this HPLC technique. Comparison of the product mixture with reference standards indicated that the ratio of L-L-L to L-D-L epimers was greater than 99.9:0.1.

(d) Disulfide Formation

In the *Prior Thiol Capture Strategy*, initial ligation of the two peptide segments occurs by formation of a mixed disulfide and, in order to optimize this process, Kemp investigated the formation of disulfides involving dibenzofuran systems.

Two general approaches to mixed disulfides were explored.[22] In each, the disulfide was generated by reaction of a free thiol with a sulfenylcarbomethoxy-derivatized thiol (-SScm), a type of reaction that was known[53] to be specific and efficient. In the first approach[22] (Scheme 29), the template thiol was derivatized with ScmCl (*cf.* **29.1**), and was then allowed to react with a free cysteine thiol [**29.1** + **29.2** → **29.3** (R = OAc)]. In the second approach[22] (Scheme 29), it was the cysteine thiol that was derivatized with ScmCl (*cf.* **29.4**), and it underwent reaction with the free template thiol [**29.4** + **29.5** (R = OAc) → **29.3** (R = OAc)]. Of the two routes, the second was found to be very much more effective, and typically gave the desired disulfide in near quantitative yields.

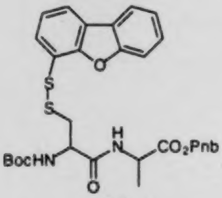
Scheme 29



Important features of the ligation step were identified by studying the kinetics of the reaction of **29.5** (R = H) with **29.4** to give **29.3** (R = H)[22] in mixtures of HFIP-acetonitrile, with and without water, and in the presence and absence of various tertiary amines. From these experiments, two general factors became apparent. First, the amine exhibited a catalytic effect on the reaction — an observation that is consistent with the view that the thiolate *anion* of **29.5** (R = H) acts as the nucleophilic species. The catalytic effect was independent of the base strength or concentration, suggesting that the actual catalyst was the conjugate base of the solvent (HFIP). The second factor revealed by the kinetic study was that an increase in solvent polarity, through the addition of water, caused a dramatic increase (*ca.* 10^4) in reaction rate.

It had been established[54] that a reaction time of 12 h was generally required to achieve approximately 90% conversion of the ligated product to the acyl transfer product and, as a result of this long reaction time, it was necessary to determine to what extent disulfide exchange occurs during the acyl transfer. To this end,[54] **30.1** (Scheme 30) was stored in DMSO for 19 h. Analysis of the product composition showed that the compound was largely (98%) unchanged and that only 2% had been converted into the corresponding symmetrical disulfides. Hence DMSO was deemed to be a suitable solvent for avoiding the problem of disulfide exchange.

Scheme 30

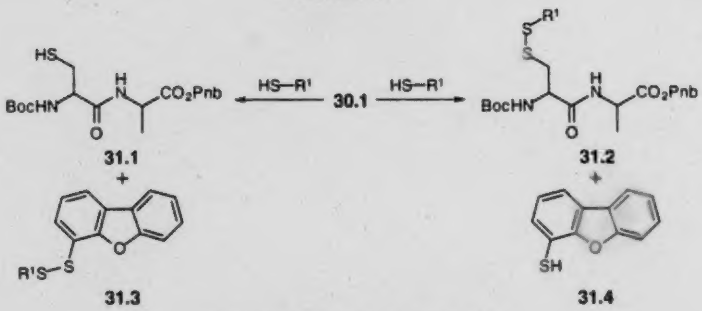


30.1

Pnb = *p*-nitrobenzyl

If the initial ligation leading to the required disulfide is not quantitative, then free thiol in the form of the template-linked *N*-terminal peptide [*cf.* **14.2** (X) = SH, Scheme 14] would be present in the reaction mixture. The effect that this thiol might have on

Scheme 31[a]

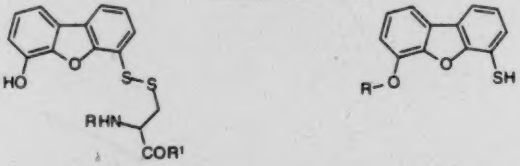


[a] R^1 = Ph or Bn; Pnb = *p*-nitrobenzyl

disulfide exchange was also examined,[54] by separately treating **30.1** with thiophenol or benzyl thiol in DMSO (Scheme 31). In each case an excess of thiol ranging from 10-100 fold was used. Under these conditions, rapid disulfide exchange occurred to give the mixed disulfides **31.3** and **31.2** in a ratio of 1:99.

In a subsequent experiment,[54] it was established that unwanted thiol-catalyzed disulfide exchange between the product **32.1** (Scheme 32) of an acyl transfer reaction and the capture thiol **32.2** used in that reaction could be suppressed almost completely by addition of the thiol scavenger silver nitrate, before acyl transfer.

Scheme 32[a]



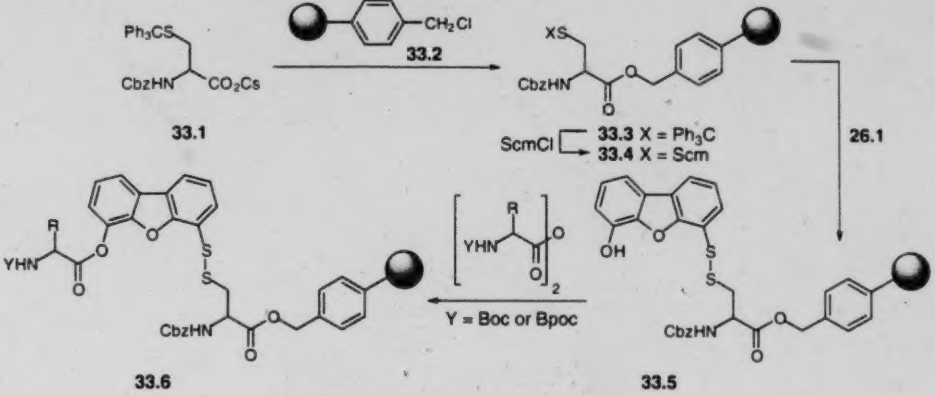
32.1 **32.2**

[a] R = CbzPFT(*t*-Bu); R' = GGAOPnb; Pnb = *p*-nitrobenzyl

(e) Synthesis of the Dibenzofuran-Linked N-Terminal Peptide Segments

Application of the *Prior Thiol Capture Strategy* requires that the *N*-terminal peptide (*cf.* **14.1**, Scheme 14) be generated in such a way that its *C*-terminus is linked to the template. For this purpose, the 4-hydroxy-6-mercaptodibenzofuran-linked solid support **33.6** was developed.[38] This support was synthesized by the route outlined in Scheme 33. Commercially available chloromethylated polystyrene resin (**33.2**) was treated with the derivatized cysteine cesium salt **33.1**, to give **33.3**. Exchange of the trityl protecting group for an Scm group (**33.3** → **33.4**) was achieved by treatment with ScmCl, and exposure of **33.4** to the mercaptodibenzofuran **26.1** under standard conditions led to disulfide **33.5**. Finally, acylation of the phenolic hydroxyl with the symmetrical anhydride of an N^{α}-blocked amino acid gave the required resin-bound disulfide **33.6**. As described below, the resin was evaluated for its ability to survive the conditions of solid phase

Scheme 33[a]

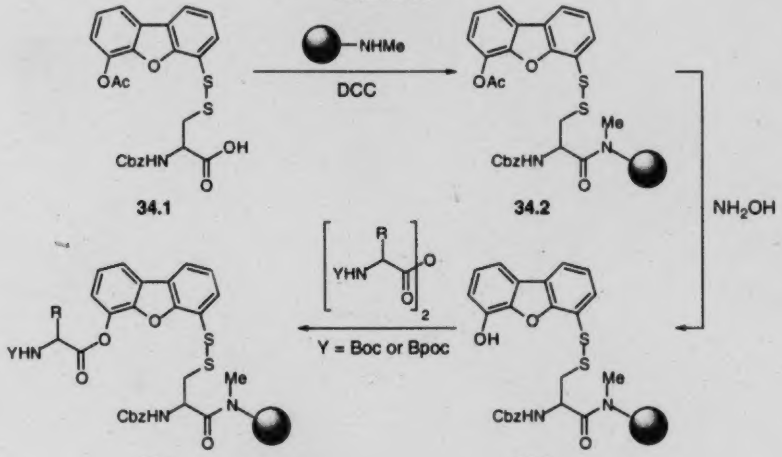


[a]● = polystyrene resin; Scm = SCO$_2$Me

synthesis.

An alternative method was also developed[22] for linking the dibenzofuran template to a solid support, and involves the use of an aminomethyl polystyrene resin. In this case (Scheme 34), compound **34.1**[55] was coupled to the resin, using DCC, to form **34.2**. Removal of the acetyl group with hydroxylamine then generated the phenolic species **34.3**,

Scheme 34[a]



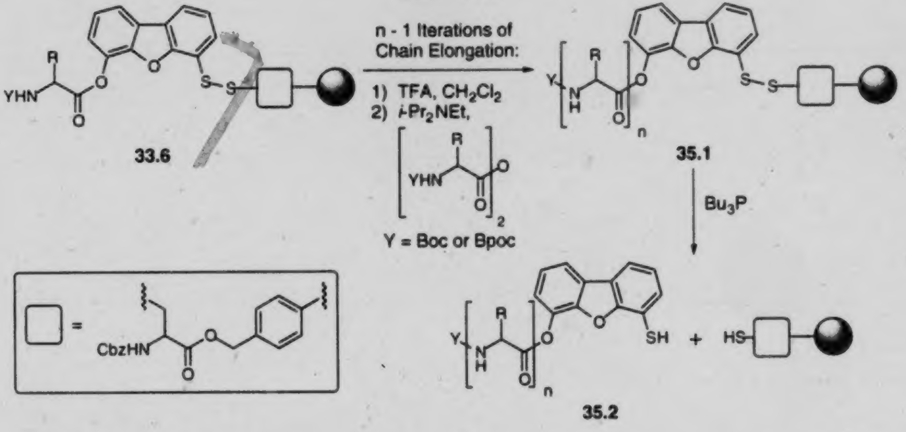
[a]● = polystyrene resin

which underwent *O*-acylation (**34.3** → **34.4**) in a manner analogous to that involved in the conversion of **33.5** into **33.6**.

The phenolic ester that anchors the peptide chain in **33.6** and **34.4** precludes the use of the fluorenylmethoxycarbonyl group (Fmoc) for N^{α}-protection, because of the requirement of a secondary amine as the deblocking reagent, but two other standard solid phase synthesis protocols are suitable for chain extension.[22,38]

The first is the combination of Boc protection for the α-amino group[38] and benzyl protection[38] for the side-chain functions. Chain elongation (Scheme 35) was initiated by treatment of **33.6** (Y = Boc) with 50% TFA in CH_2Cl_2 to give the corresponding TFA salt. This salt was then treated with an appropriately protected aminoacyl anhydride in the presence of *i*-Pr_2NEt, giving a product elongated by one residue. The sequence was repeated until the desired peptide (**35.1**) had been assembled. Treatment with tributylphosphine[38] then served to reductively cleave the disulfide bond, giving the

Scheme 35



protected *N*-terminal peptide **35.2**, already derivatized at its *C*-terminus as a 4-hydroxy-6-mercaptodibenzofuran ester. The peptide could also be obtained in fully unprotected form by simply effecting global deprotection of the side-chains prior to resin cleavage.

The second solid phase protocol[38] employed Bpoc (*p*-$PhC_6H_4CMe_2OCO$-)

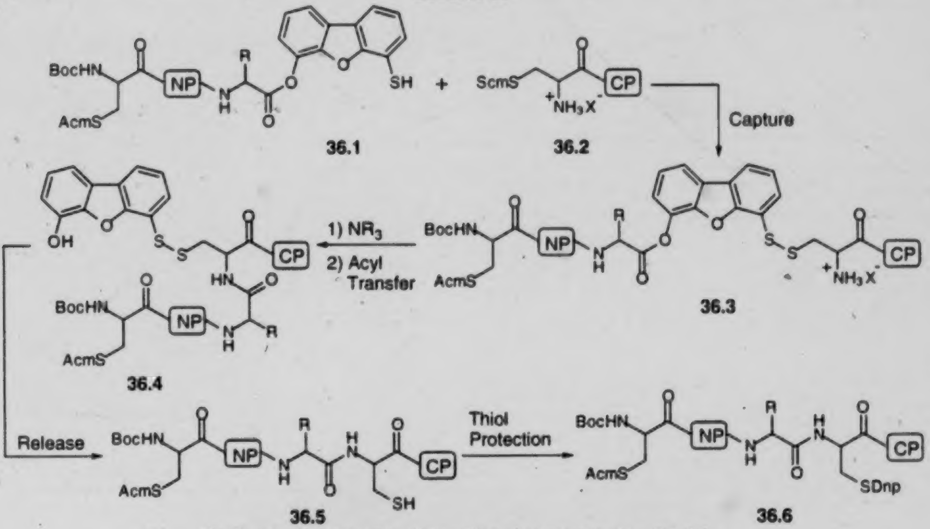
protection of the α-amino group (Y = Bpoc, Scheme 35), combined with *t*-butyl[22] and Boc protection[38] for the side-chain functions. In this case, chain elongation was carried out as described above, with the exception that the Bpoc protecting group was removed using 0.5% TFA in the presence of 1% thioanisole.

(*f*) *Cysteine Thiol Protection and a Modified Prior Thiol Capture Strategy*

In order to implement the *Prior Thiol Capture Strategy*, it was necessary to have available a variety of protecting groups for cysteine thiol residues, both internal and terminal.[22] Protection of this type is required because there is no general method[56] for forming a disulfide selectively from one thiol in the presence of others. Aside from the cysteine side-chains, no other protection of substituents in either peptide segment is required.[57]

The approach that was developed[22,56,58] to deal with selective protection of cysteine residues is summarized in Scheme 36.[56] The thiol group of the *N*-terminal

Scheme 36[a]

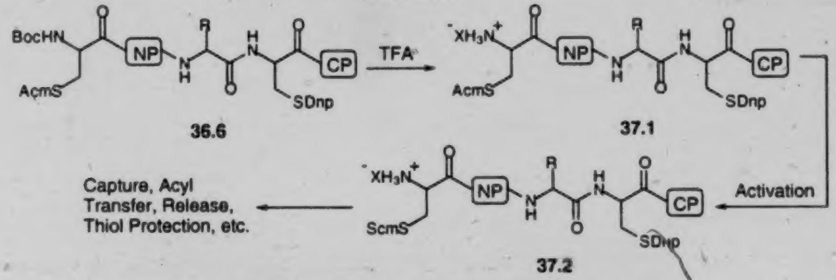


[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide; X = CF_3CO_2; Acm = $CH_2NHCOMe$; Scm = SCO_2Me; Dnp = 2,4-dinitrophenyl

cysteine of the *C*-terminal peptide **36.2** is protected with a sulfenylcarbomethoxy (Scm) group, and the *N*-terminal cysteine thiol of the dibenzofuran-linked peptide **36.1** is protected as an acetamidomethyl (Acm) thioether.[59] Compound **36.2** undergoes ligation with the *N*-terminal peptide, by nucleophilic displacement of the Scm moiety by the template thiol, to generate the acyl transfer intermediate (**36.1** + **36.2** → **36.3**). Treatment with an amine initiates acyl transfer, so as to couple the two peptide segments and give compound **36.4**. Cleavage of the template from **36.4** is then followed by thiol protection (treatment with 2,4-dinitrofluorobenzene in a bicarbonate buffer) as the 2,4-dinitrophenylsulfenyl (Dnp) derivative **36.6** (**36.4** → **36.5** → **36.6**).

The above series of protecting groups used for the cysteine thiol functions was chosen for the following reasons. First, both the Dnp- and the Acm-protected thiols are inert to attack by the nucleophilic template thiol, and so no complications would result from formation of disulfides other than the one required for acyl transfer. The second reason is that, unlike the Dnp-protected thiol, the Acm-protected thiol is readily convertible into the corresponding Scm form,[60] which *is* susceptible to nucleophilic attack by the template thiol. Discrimination between the protected thiol groups is required so that, once the coupled product has been liberated from the template (**36.4** → **36.5**), the free thiol group can be converted into its Dnp-protected form (**36.5** → **36.6**) and, following removal of the N^{α}-Boc group (**36.6** → **37.1**, Scheme 37), the Acm-protected *N*-terminal cysteine

Scheme 37[a]



[a] NP = *N*-terminal peptide; CP = *C*-terminal peptide; X = CF_3CO_2; Acm = $CH_2NHCOMe$; Scm = SCO_2Me; Dnp = 2,4-dinitrophenyl

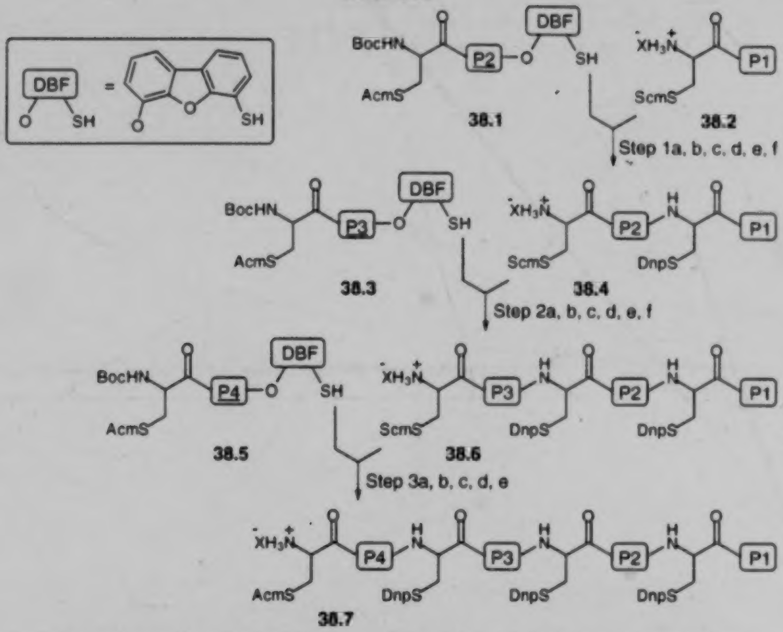
thiol can then be activated by conversion into its Scm derivative (**37.1** → **37.2**) — a sequence of steps that would set the stage for a second ligation between **37.2** and a new template-linked *N*-terminal peptide (*cf.* **36.1**), marking the beginning of a new cycle of peptide coupling. In principle, the whole sequence can be repeated as many times as desired.

(g) Application to Peptide Synthesis

The *Prior Thiol Capture Strategy* has been applied to the synthesis of several medium-sized peptides. One of these is the 29-residue *C*-terminal segment of the protein *basic pancreatic trypsin inhibitor* (BPTI).[22] This segment corresponds to residues 30-58 of the native protein. The synthesis of this peptide was the first major test of the *Prior Thiol Capture Strategy*, and was effected by carrying out three cycles of the process outlined in Schemes 36 and 37, but with one modification. Instead of using *N*-terminal peptide segments with protecting groups only on the *N*-terminal nitrogen and cysteine residues (*cf.* **36.1**), fully protected segments were used. The non-cysteinyl protecting groups were all TFA-labile and so, after Dnp protection of the new thiol group (*cf.* **36.5** → **36.6**), treatment with TFA also removed the non-cysteine side-chain protecting groups (both SAcm and SDnp are stable under these conditions). This reaction gave a new *C*-terminal peptide, protected only on its cysteine side-chains, which could undergo activation (*cf.* **37.1** → **37.2**) and enter into another coupling cycle.

The synthesis of the peptide in question was carried out as summarized in Scheme 38. In the first step, the activated *C*-terminal peptide **38.2** underwent capture by the fully protected segment **38.1** (step 1a), to give the ligation intermediate. Acyl transfer (step 1b) then occurred, and was followed by trialkylphosphine-mediated template cleavage (step 1c), Dnp protection of the liberated thiol (step 1d), deprotection of the *N*-terminal nitrogen and non-cysteine amino acid side-chains (step 1e) and, finally, activation of the *N*-terminal cysteine thiol by conversion into its Scm derivative (step 1f). The product of this coupling

Scheme 38[a]



[a] a = capture; b = acyl transfer (*i*-Pr_2NEt); c = template cleavage (Et_3P); d = thiol protection (SH → SDnp); e = deprotection (TFA); f = activation (SAcm → SScm); P1 = GGA; P2 = MR(Pmc)T(*t*-Bu); P2 = deprotected form of P2; P3 = R(Pmc)AK(Boc)R(Pmc)NNFK(Boc)-S(*t*-Bu)AE(*t*-Bu)D(*t*-Bu); P3 = deprotected form of P3; P4 = QT(*t*-Bu)FVY(Dnp)GG; P4 = QTF-VY(Dnp)GG; X = CF_3CO_2; Acm = $CH_2NHCOMe$; Scm = SCO_2Me; Dnp = 2,4-dinitrophenyl; Pmc = pentamethylchromane

cycle was the activated, cysteine-protected octapeptide **38.4**, which corresponds to residues 51-58 of native BPTI. During the second cycle of the synthesis, this segment was coupled to the template-derivatized 13-residue segment (**38.3**), corresponding to residues 38-50 of the native protein. The product of this second coupling cycle was the 21-residue peptide **38.6**. In the third and final cycle, the activated segment **38.6** was combined with the octapeptide **38.5**, to give the target peptide **38.7**, protected only on its cysteine side-chains. Overall yields for each of the six-step cycles were between 50% and 75%. At high pH a second product was obtained resulting from intramolecular acyl transfer to the ε-amino group of the lysine residue at site 3 of peptide P3, but no acyl transfer to the second lysine of P3 at site 8 was detected. It is not unexpected that at high pH an ε-amino group is acylated since, under these conditions, the lysine ε-amino group is as abundant as the *N*-terminal cysteine α-amino group and, as reflected in the higher pK_a of its conjugate acid, it should be a better nucleophile. The fact that the more distal ε-amino group is not acylated indicates an upper limit to the distance over which intramolecular acyl transfer can occur, and a model study has shown that, within this distance, selectivity of transfer can be very largely controlled by proper pH adjustment.[62]

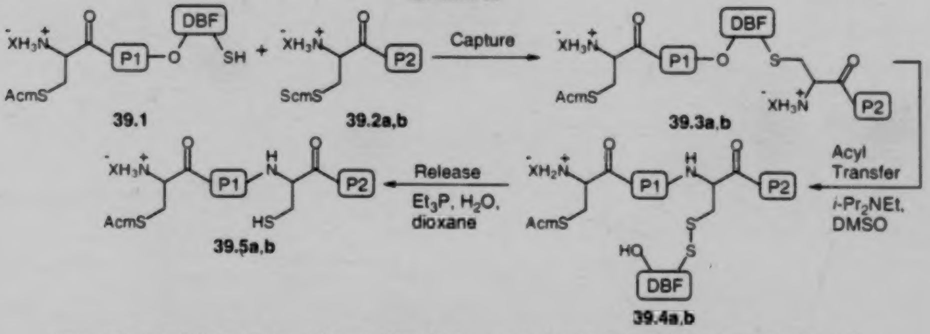
Subsequent to the above synthesis, the *Prior Thiol Capture Strategy* was applied to the synthesis of a 25- and a 39-residue peptide.[58] In these cases, however, peptide segments that were fully deprotected, except on their cysteine residues, were used. The 39-residue peptide corresponded to residues 26-63 of the 63-amino acid *ColE1 repressor of primer protein*, but had isoleucine-37 replaced by leucine, and had a cysteine residue attached to its *N*-terminus. This peptide was synthesized by coupling a 13-residue segment corresponding to amino acids 26-37 of the native protein (but with the additional *N*-terminal cysteine) to a 27-residue segment corresponding to amino acids 27-63 of the native protein and having the leucine-37 substitution. The 25-residue peptide was synthesized by coupling the same 13-residue *N*-terminal fragment used for the 39-residue peptide with a segment corresponding to residues 52-63 of the native protein.

Each of the *C*-terminal segments used for the synthesis of these two peptides was generated using standard solid phase procedures. Synthesis of the common *N*-terminal fragment, however, made use of the special dibenzofuran-linked solid phase resin (**34.3**) described above (see Scheme 34).

The synthesis of the 25- and 39-residue peptides is outlined in Scheme 39. In each case, the coupling cycle was initiated by reacting the template-linked *N*-terminal peptide **39.1** with the appropriate activated *C*-terminal peptide (**39.2a,b**), to give the ligated product (**39.1** + **39.2a** → **39.3a**; **39.1** + **39.2b** → **39.3b**).

The ligated product so obtained was then treated with base in order to initiate acyl transfer, and this process gave rise to compounds **39.4a,b**. At this stage reductive

Scheme 39[a]



[a] P1 = LNELDADEQADL; P2 = LARFGDDGENL (for **a** series); P2 = ESLHDHADELYRSC(S*t*-Bu)LA-RFGDDGENL (for **b** series); P2 = ESLHDHADELYRSCLARFGDDGENL (for **39.5b**); X = CF_3CO_2

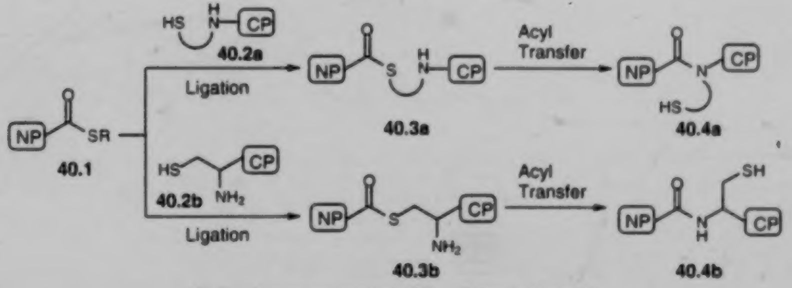
cleavage was carried out in order to remove the template and generate the desired peptide segments **39.5a,b**. (In the case of template cleavage from **39.4b**, the internal cysteine *t*-butyldisulfide protecting group was also cleaved.) In this way, the 25-residue peptide **39.5a** and the 39-residue peptide **39.5b** were obtained in 82% and 80% yield, respectively.

1.5 Native Chemical Ligation Strategies

A third ligation-based approach to peptide segment coupling is the *Native Chemical Ligation Strategy*, which was developed by Kent.[63] In this strategy, ligation of the segments prior to peptide bond formation occurs through a thioester link between the two segments to be coupled. The thioester is formed by exchange between a thiol and a thioester, a type of reaction that has been studied extensively[64] and is known to proceed in a highly chemoselective manner. Kent's approach can be outlined in a general way by reference to Scheme 40. Here, the thiol is part of the *N*-terminal amino acid residue of the *C*-terminal peptide segment (**40.2a,b**), and the thioester is simply a derivatized form of the carboxyl terminus of the *N*-terminal peptide (**40.1**). Ligation occurs when the two components are mixed in solution, and the reaction generates an intermediate (**40.1 +**

40.2a → **40.3a**; **40.1** + **40.2b** → **40.3b**) that is capable of undergoing intramolecular *S*→*N* acyl transfer. The transfer in this case gives rise to a coupled product (**40.4a,b**) which, depending on the specific approach used, may or may not require further manipulation.

Scheme 40[a]



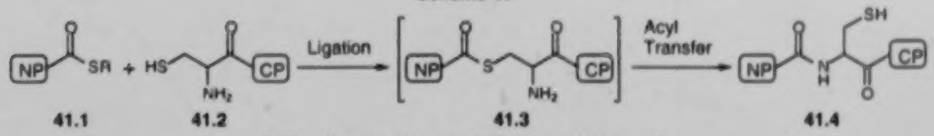
[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

From Kent's original ideas on *Native Chemical Ligation* two related procedures have emerged. The main distinction between them is the source of the nucleophilic thiol involved in the ligation step. This ligation determines the site at which coupling occurs between the two peptide segments. In one approach, the source of the thiol is an *N*-terminal cysteine residue, and coupling occurs between that cysteine and some other amino acid. In the other approach, an *N*-ethanemercapto- (*N*-CH_2CH_2SH) or *N*-oxyethanemercapto- (*N*-OCH_2CH_2SH) derivatized glycine or alanine residue provides the nucleophilic sulfur and, as a result, coupling occurs between that glycine or alanine and some other amino acid.

1.5.1 Ligation by Thioester Exchange Involving Cysteine

In the first of the *Native Chemical Ligation* approaches,[63] an *N*-terminal cysteine on the *C*-terminal peptide provides the thiol substituent that is required for ligation. The ligation is chemoselective so no protecting groups should be required on either peptide segment. The general strategy, which has also been applied in another context (see Scheme 62), is outlined in Scheme 41. The principle is that ligation occurs when the thioester moiety of the *N*-terminal peptide (**41.1**) undergoes nucleophilic attack by the thiol group of the *N*-terminal cysteine of the other peptide (**41.1** + **41.2** → **41.3**). This process gives rise to an acyl transfer intermediate (**41.3**) which is not isolated, but which undergoes spontaneous intramolecular acyl transfer to generate the coupled product directly (**41.3** → **41.4**). In this case no further manipulation is required.

Scheme 41[a]



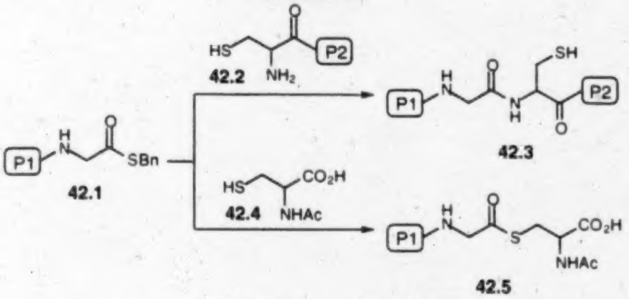
[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

(a) *Mechanistic and Practical Aspects*

Intramolecular acyl transfer in the cysteine-based *Native Chemical Ligation Strategy* must proceed via a five-membered transition state from an intermediate (*cf.* **41.3**) that is not isolated. Indirect evidence has been obtained for the proposed intermediate in two separate experiments. In the first, the thioester-derivatized *N*-terminal peptide **42.1** (Scheme 42) was treated with *N*-acetylcysteine (**42.4**).[63] Ligation proceeded to give **42.5**, but this compound cannot undergo acyl transfer because the amino group of the cysteine is blocked, and so **42.5** was isolated in the form shown. When the same *N*-terminal peptide (**42.1**) was treated with a fully deprotected *C*-terminal peptide having an *N*-terminal cysteine, the expected coupling product was obtained (**42.1** + **42.2** → **42.3**).

Additional evidence for the proposed intermediate was obtained indirectly from an experiment that was also used to established the requirement for cysteine at the *N*-terminus of the *C*-terminal peptide.[63] In this work, a thioester-derivatized *N*-terminal peptide (*cf.* **41.1**) was combined with a 10-fold molar excess of the *C*-terminal peptide Leu-

Scheme 42[a]

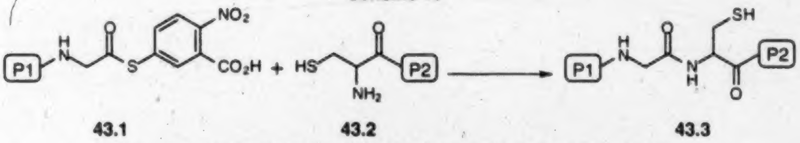


[a] P1 = LYRA; P2 = RAEYS

enkephalin[65] which has, at its *N*-terminus, a tyrosine residue. No reaction occurred. However, when the same *N*-terminal peptide was treated with a different *C*-terminal peptide, having a cysteine residue at its *N*-terminus (*cf.* **41.2**), rapid coupling took place. These observations implied not only that the proposed intermediate did indeed form, but also that the ligation proceeds in a highly chemoselective manner.

Coupling between **42.1** and **42.2** to give **42.3** (Scheme 42) proceeds readily in solution at pH 6.8, but very slowly when the pH is below 6.0.[63] In addition, when coupling between **43.1**, made as described below, and **43.2** (Scheme 43) was carried out

Scheme 43[a]



[a] P1 = MscCTETLQNAHSMALPALEPSTRYWARVRVRT; P2 = TGYN-GIWSEWSEARSWDT; Msc = [2-(methanesulfonyl)ethyloxy]carbonyl

at pH 7.0, reaction was essentially complete after only 5 min. However, when the same components were coupled at pH 5.0, the reaction was only about 50% complete after 10 min.[63] These observations are qualitatively in accord with the plausible mechanism that the thiolate is the reactive nucleophile.

The coupling of **43.1** and **43.2** showed the expected influence of the sulfur leaving group on the ligation process. The former compound was made from the corresponding thioacid by treatment with 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent). This transformation is assumed to involve the intermediacy of a short-lived disulfide [$-C(O)SSC_6H_3(NO_2)CO_2H$], which rapidly affords the thioester **43.1**.[66] The coupling experiment confirmed that the nature of the thioester leaving group influences the rate of reaction; the initial investigations into coupling by *Native Chemical Ligation* had employed benzyl thiol as the leaving group (*cf.* **42.1**) but, in the present case, 5-mercapto-2-nitrobenzoic acid serves in that role, and the result was much faster coupling.[63] In a subsequent experiment, addition of thiophenol to a coupling reaction that used a benzyl thiol leaving group also enhanced the rate of the coupling.[67] Presumably, the increase is due to the fact that the PhS group replaces BnS prior to ligation and, therefore, provides a better leaving group. This technique of *in situ* conversion to a more reactive species has been used in the synthesis of peptides with over 100 amino acid residues.[67]

A practical aspect of the coupling reaction is the need to suppress oxidation of free cysteine thiol groups. Oxidation of this type renders the ligation step ineffective, since it decreases the nucleophilic character of the sulfur atom on the *N*-terminal cysteine, and prevents ligation. It was established,[63] fortunately, that this problem could easily be circumvented by carrying out the ligation in the presence of an excess of the thiol corresponding to the thioester leaving group.

Despite the fact that coupling in the *Native Chemical Ligation Strategy* is carried out with completely unprotected peptide segments, no incompatibility with the presence of other cysteine residues was apparent.[63] Presumably, this results from the fact that inter- or intramolecular reaction of cysteine residues, other than the intended one, with the thioester is unproductive and also reversible. The necessary juxtaposition of the required groups for acyl transfer can realistically be achieved only by reaction of the *N*-terminal cysteine of the *C*-terminal peptide (*cf.* **41.2**) with the thioester substituent of the *N*-terminal peptide (*cf.*

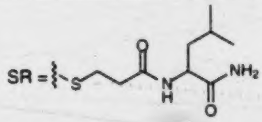
41.1). Reaction of other cysteines with the thioester will undoubtedly occur, but does not result in a system in which an amino group and the acyl group are sufficiently close to permit rapid acyl transfer. Instead, the products of such undesired ligation would be expected to react with the excess of the thiol corresponding to the leaving group, and revert back to starting material. Eventually, a productive ligation occurs, and is followed by the desired acyl transfer.

An investigation into the effect of the *C*-terminal amino acid of the *N*-terminal segment on the rate of coupling in the *Native Chemical Ligation Strategy* has been carried out. This was done by examining coupling of the *N*-terminal segment LYRAX-SR and the *C*-terminal segment CRANK.[68] In this case, X-SR is an amino acid thioester[69] corresponding to one of the 20 naturally-occurring amino acids. Each of the 20 possible thioesters was examined and the amino acids were then grouped according to the time required for the coupling to reach completion (see Table 8). Ligation proceeds efficiently in all cases, except when the thioester-derivatized amino acid is β-branched or is proline. Interestingly, when the thioester was a histidine or cysteine residue, coupling occurred at approximately the same rate as with the sterically unhindered glycine residue. It was

Table 8[68]

Effect of *N*-terminal residue (X) of *C*-terminal Segment on Coupling of LYRAX-SR and CRANK by *Native Chemical Ligation*

X	Coupling Time (h)	X	Coupling Time (h)	X	Coupling Time (h)	X	Coupling Time (h)
Gly	≤ 4	Ala	≤ 9	Arg	≤ 24	Ile	≥ 48
Cys	≤ 4	Met	≤ 9	Asn	≤ 24	Leu	≥ 48
His	≤ 4	Phe	≤ 9	Asp	≤ 24	Pro	≥ 48
		Trp	≤ 9	Gln	≤ 24	Thr	≥ 48
		Tyr	≤ 9	Glu	≤ 24	Val	≥ 48
				Lys	≤ 24		
				Ser	≤ 24		

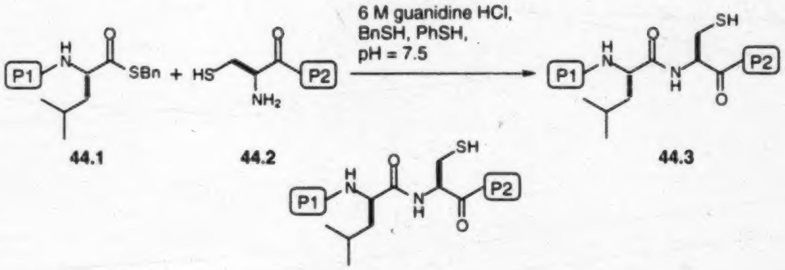


suggested that this result might be due to a catalytic effect displayed by the side-chain (thiol or imidazole) on the rate-limiting thioester exchange.

(b) Extent of Epimerization

The extent, if any, of epimerization during the coupling process was monitored in the following way.[70] The model peptides **44.1** and **44.2** were prepared and allowed to react in 6 M guanidine hydrochloride at pH 7.5, in the presence of 1% benzyl thiol and 3% thiophenol (Scheme 44), to give **44.3**. The leucine epimer (**44.4**) of this compound was also made, but by standard solid phase peptide synthesis. HPLC conditions were found that allowed baseline resolution of the two epimers (**44.3** and **44.4**) and, when the product of the ligation reaction was analyzed, no epimerization product was detected; and if any had occurred it must have been to the extent of <1%.

Scheme 44[a]

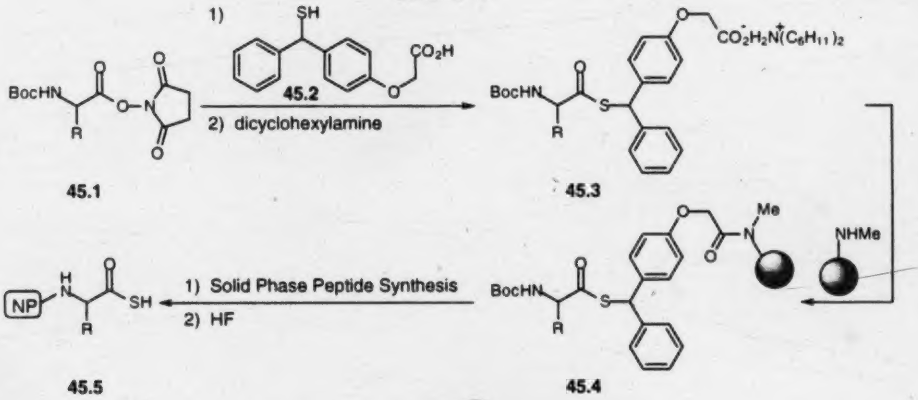


[a] P1 = PACTLEYRP; P2 = GSDNKTYGN

(c) Synthesis of Peptide Segments

The peptide segments used for the cysteine-based *Native Chemical Ligation Strategy* were obtained by solid phase peptide synthesis. The synthesis of the *C*-terminal peptides was straightforward, given that they possessed no unusual features. However, synthesis of the *N*-terminal peptides — which are derivatized at their carboxyl termini as thioesters — required some developmental work. The thioesters could be obtained readily from the corresponding thioacids by reaction with an excess of benzyl bromide in 6 M guanidine hydrochloride at pH 4.6 for the benzyl esters, or with 5,5'-dithiobis(2-nitrobenzoic acid) in 8 M urea at pH 4.0 for the 5-mercapto-2-nitrobenzoic acid thioesters.[63] In order to obtain the starting thioacid-derivatized peptide, however, an optimized form of solid phase synthesis, based on the thioester-linked solid support **45.4** (Scheme 45), had to be used.[70-72] This support was generated[71] by reacting an appropriately protected *N*-hydroxysuccinimide amino acid ester (**45.1**) with the benzylic thiol **45.2**[73] to give, after treatment with dicyclohexylamine, salt **45.3**. This salt was then coupled to an aminomethyl polystyrene resin,[72] generating the required solid support (**45.3** → **45.4**). At this point, standard solid phase peptide synthesis was carried out in order to produce the required peptide segment, and then treatment of the resulting resin-bound peptide with HF cleaved the product from the support, and released the required peptidyl thioacid (**45.5**).

Scheme 45[a]

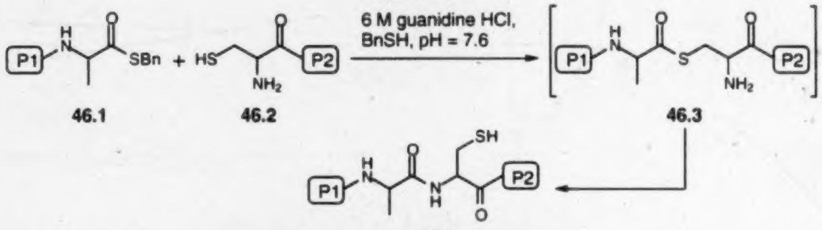


[a]NP = *N*-terminal peptide; ● = polystyrene resin

(d) *Application to Peptide Synthesis*[74]

One of the first applications of the cysteine-based *Native Chemical Ligation Strategy* was in the synthesis of a *human interleukin 8* mutant (**46.4**, Scheme 46).[63] In this particular mutant, the histidine residue at position 33 was substituted by an alanine residue.[63] The protein was 72 amino acids in size, and contained a total of four cysteine residues. Its synthesis was achieved[63] by coupling the 33-residue *N*-terminal peptide segment **46.1** and the 39-residue *C*-terminal peptide segment **46.2**. Significantly, a total of 18 out of the 20 genetically encoded amino acids are represented in these segments. The site of coupling was chosen to be between cysteine 34 and alanine 33. The two peptide segments were allowed to react in 6 M guanidine hydrochloride at pH 7.6, in the presence of an excess of benzyl thiol, and underwent ligation to generate the acyl transfer intermediate (**46.1** + **46.2** → **46.3**). Intramolecular *S*→*N* acyl transfer (**46.3** → **46.4**) then gave the coupled product in approximately 60% yield after 48-72 h.

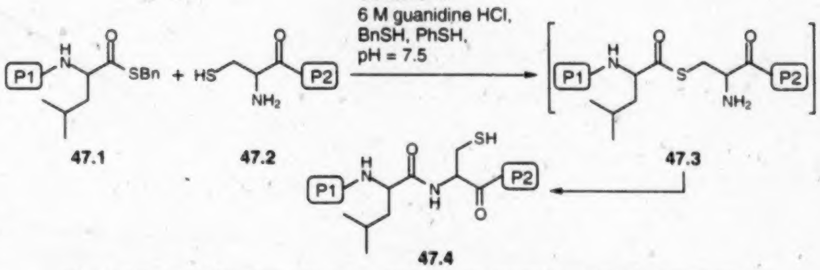
Scheme 46[a]



[a]P1 = SAKELRCQCIKTYSKPFHPKFIKELRVIESGP;
P2 = ANTEIIVKLSDGRELCLDPKENWVQRVVEKFLKRAENS

A second application of the cysteine-based *Native Chemical Ligation Strategy* was in the synthesis of the peptide corresponding to residues 6-56 of *turkey ovomucoid third domain*.[70] The synthesis of this peptide was achieved by coupling the 18-residue *N*-terminal peptide **47.1** (Scheme 47) and the 33-residue *C*-terminal peptide **47.2**. The coupling site was chosen to be between cysteine-24 and leucine-23. Peptides **47.1** and **47.2**, the former made[70] by the procedure summarized in Scheme 45, and the latter manually synthesized by an optimized[70] solid phase method based on Boc chemistry, underwent smooth ligation in 6 M guanidine hydrochloride at pH 7.5, in the presence of

Scheme 47[a]

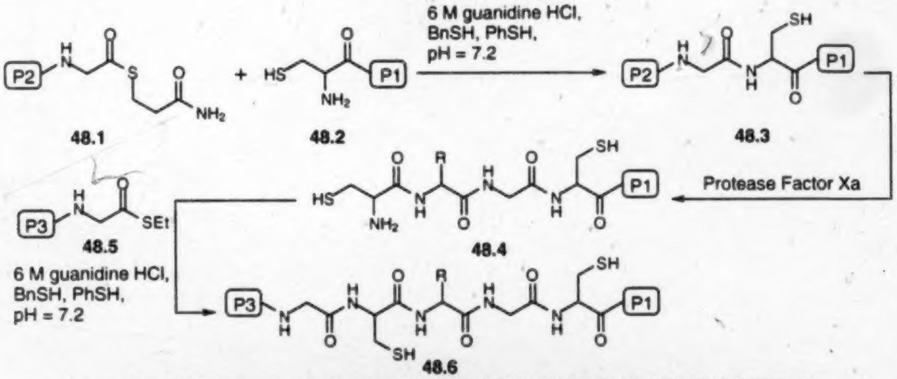


[a]P1 = VDCSEYPKPACTLEYRP; P2 = GSDNKTYGNKCNFCNAVVESNGTLTLSHFGKC

1% benzyl thiol and 3% thiophenol, to give the acyl transfer intermediate **47.3**, which then rearranged to the coupled product **47.4**. Peptide **47.4** was isolated from the mixture after 36 h, and purified by reverse-phase HPLC. The isolated yield was typically about 56%.

One final noteworthy application of the *Native Chemical Ligation Strategy* is the formation of the 164-residue peptide **48.6** (Scheme 48).[76] In this example, the peptide segments were recombinantly derived, and so the approach serves to demonstrate the versatility, as well as the power of *Native Chemical Ligation* for coupling large segments. Since two sequential couplings were used, the potential for iteration was also demonstrated. The first coupling was between the 105-residue *C*-terminal peptide **48.2**,

Scheme 48[a]



[a]P1 = LEKHSWYHGPVSRNAAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHYRINTA-SDGKLYVSSESRFNTLAELVHHHSTVADGLITTLHYPAPKRGIHRD; P2 = RGKIEGRCK(Dns); P3 = MLFVALYDFVASGDNTLSITKGEKLRVLGYNHNGEWAEAQTKNGQGWVPSNYITPV; R = $(CH_2)_4$NH-Dns; Dns = [5-(dimethylamino)-1-naphthyl]sulfonyl

and the thioester-derivatized decapeptide **48.1**, and was achieved by storing the two components for 96 h in the presence of 6 M guanidine hydrochloride at pH 7.2, along with 1.5% benzyl thiol and 1.5% thiophenol (**48.1** + **48.2** → **48.3**). In this case, the decapeptide served as a masked form of the thioester-derivatized tripeptide CK(Dns)$GSCH_2CH_2CONH_2$. The additional seven residues of the decapeptide functioned as a protecting group for the *N*-terminal cysteine, and were removed enzymatically after the first coupling, by exposure to protease factor Xa (**48.3** → **48.4**). At this point, the stage was set for the second coupling, which was achieved by treating **48.4** with the 56-residue, thioester-derivatized peptide **48.5** under the same conditions used for the first coupling (**48.4** + **48.5** → **48.6**). The overall yield for the entire process was 28%.

(e) Application to the Solid Phase (Solid Phase Chemical Ligation)

The *Native Chemical Ligation Strategy* has recently been extended to allow coupling in the solid phase.[77] This can be accomplished using either of two separate approaches which Kent calls *Solid Phase Chemical Ligation* techniques. In the first approach, the thioester-derivatized *N*-terminal peptide is anchored to a resin through its *N*-terminus and is treated with a *C*-terminal peptide which has at its *N*-terminus a cysteine residue. Ligation and acyl transfer then proceed in the usual manner, with the exception that the coupled product remains attached to the solid support. Thus, peptide synthesis occurs on the solid support in the *N* to *C* direction. In the second approach, coupling is carried out in the *C* to *N* direction by first attaching a *C*-terminal peptide bearing an *N*-terminal cysteine residue to the solid support via its *C*-terminus, and then treating the product with a thioester-derivatized *N*-terminal peptide. Again, ligation and acyl transfer proceed as usual, but result in a resin-bound coupled product.

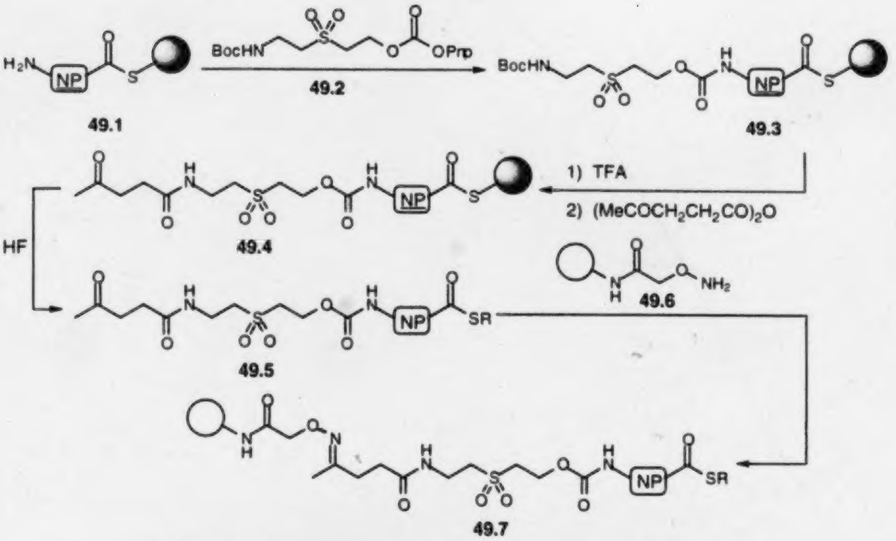
During the development of this solid phase segment coupling approach, Kent was able to incorporate into the methodology the option of carrying out the process in an iterative fashion. In this case, the product of the first coupling reaction becomes the resin-bound peptide used in the second coupling cycle, and the product of that cycle, in turn, is then the resin-bound component in the next cycle. The iterative coupling requires that the non resin-bound peptide, which possesses both an *N*-terminal cysteine residue and a *C*-terminal thioester, be introduced in a masked form in which one or the other of its termini (which one would depend on which of the two approaches was being used) is suitably masked. Protection of this type is necessary to avoid unwanted cyclization or, possibly, polymerization of the non resin-bound peptide. Following ligation and acyl transfer, the protecting group is removed, and the product is ready to enter into another coupling cycle (see later).

(i) Solid Phase Chemical Ligation in the N to C direction[77]

Solid Phase Chemical Ligation in the *N* to *C* direction requires that the original *N*-terminal peptide be attached to the solid support. To achieve this, routine stepwise solid phase peptide synthesis was used to generate the desired *N*-terminal peptide (**49.1**, Scheme 49) but, prior to deprotection and resin cleavage, the unprotected α-amino residue was derivatized with *p*-nitrophenyl carbonate **49.2**[78] to give the Boc-protected amine **49.3**. Removal of the Boc group was followed by acylation,[79] and gave the keto derivative **49.4**. This species was subjected to global deprotection and resin cleavage (**49.4** → **49.5**), resulting in a free unprotected peptide possessing a cleavable linker at its amino terminus.[80] The ketonic group of the linker was employed, via oxime formation, as the site of attachment to a water-compatible, cellulose-based aminooxy acetic acid-derivatized resin (**49.6**), resulting in formation of **49.7**.

At this point *Native Chemical Ligation* could be carried out between the resin-bound thioester **49.7** and the first *C*-terminal segment (**50.1**, Scheme 50). In this process unwanted side reactions involving the *C*-terminus of the non resin-bound peptide were suppressed by introducing this peptide in the form of a thiocarboxylate ion. The *C*-terminal

Scheme 49[a]

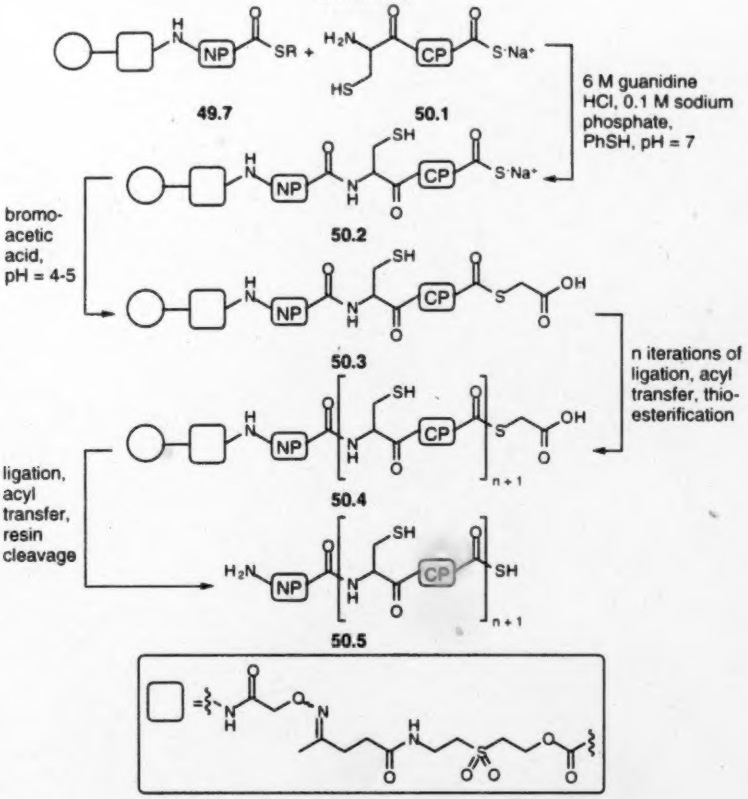


[a]NP = protected *N*-terminal peptide; NP = unprotected *N*-terminal peptide; Pnp = *p*-nitrophenyl; ● = polystyrene resin; ○ = cellulose resin

carbonyl in this form was found[77] to be sufficiently unreactive towards the *N*-terminal cysteine thiol, provided the thioester-containing *N*-terminal peptide (**49.7**) was present. However, in the absence of the thioester, detectable cyclization of the non resin-bound peptide did occur.[77] Ligation and acyl transfer (**49.7 + 50.1 → 50.2**) were carried out at pH 7 in the presence of 1% thiophenol. Once coupling was complete, the pH was lowered to 4-5 and the thiocarboxylate was converted into a thioester by treatment with bromoacetic acid (**50.2 → 50.3**). Excess reagent was washed away, and the pH was returned to 7 in preparation for another coupling cycle. Following the desired number of iterations, the peptide was liberated from the solid support by cleavage with aqueous sodium hydroxide at pH 12-14 (**50.3 → 50.4 → 50.5**), and then purified by HPLC.

Solid Phase Chemical Ligation in the *N* to *C* direction has been applied to the synthesis of three peptides ranging in size from 68 to 115 amino acid residues.[77] In each case, two iterations of the coupling cycle were employed to reach the target, and so the

Scheme 50[a]


49.7
50.1
6 M guanidine HCl, 0.1 M sodium phosphate, PhSH, pH = 7
50.2
bromo-acetic acid, pH = 4-5
50.3
n iterations of ligation, acyl transfer, thio-esterification
50.4
ligation, acyl transfer, resin cleavage
50.5
NP
CP

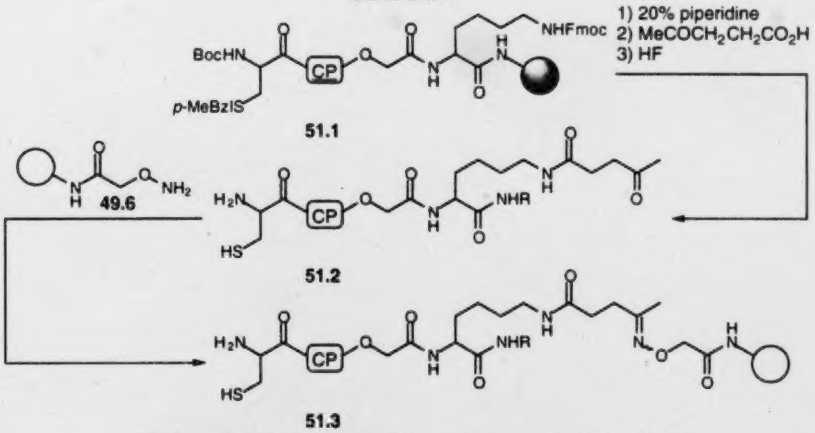
[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide; ○ = cellulose resin

process involved the use of one resin-bound *N*-terminal peptide and two non resin-bound *C*-terminal peptides.

(ii) Solid Phase Chemical Ligation in the C to N direction[77]

Preparation of the resin-linked *C*-terminal peptide for use in *Solid Phase Chemical Ligation* in the *C* to *N* direction is outlined in Scheme 51. The desired peptide segment was built up using standard stepwise solid phase techniques from a support incorporating a linker which contained a carboxyamidomethyl group attached to an ε-amino Fmoc-protected lysine residue. Once the desired peptide (**51.1**) had been generated, the Fmoc group was removed by treatment with 20% piperidine, and the liberated ε-amino group was acylated with levulinic acid. Global deprotection using HF was then effected and the resulting *C*-terminal-derivatized peptide **51.2** was isolated and purified by HPLC. The ketone functional group was used to anchor the newly-formed *C*-terminal peptide to a water-compatible, cellulose-based aminooxy acetic acid-derivatized resin via formation of an oxime (**51.2** + **49.6** → **51.3**).

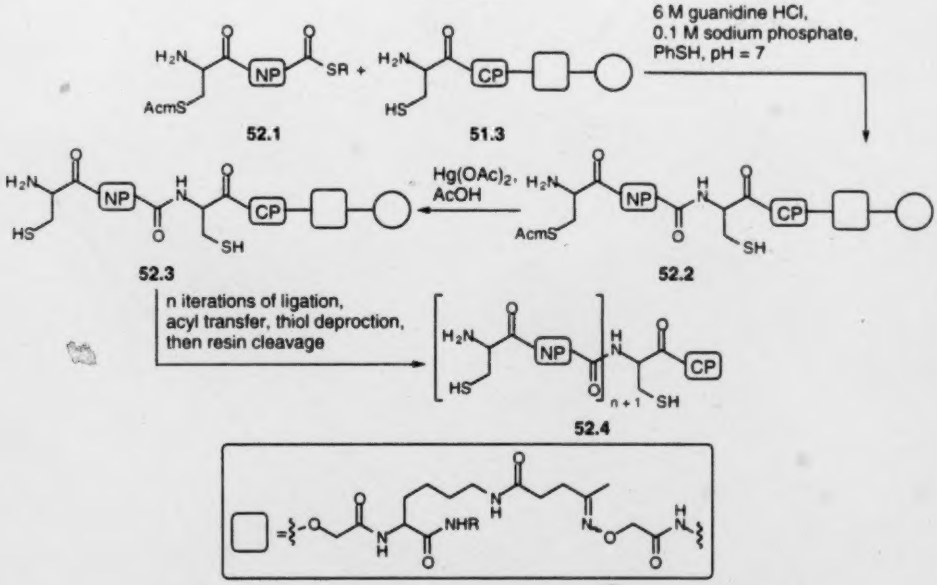
Scheme 51[a]



[a]CP = protected *C*-terminal peptide; CP = unprotected *C*-terminal peptide; ● = polystyrene resin; ○ = cellulose resin

The non resin-bound, thioester-derivatized *N*-terminal peptide **52.1** (Scheme 52) used in the ligation/acyl transfer reaction was obtained by standard solid phase techniques and contained an *N*-terminal Acm-protected cysteine to prevent undesired side reactions. Ligation and acyl transfer (**52.1** + **51.3** → **52.2**) were carried out at pH 7 in the presence of 1% thiophenol and, once complete, excess reagents were washed away and the Acm group was removed by treatment with $Hg(OAc)_2$ and AcOH (**52.2** → **52.3**). The product so obtained could then be used in subsequent coupling cycles and, once the final peptide had been generated, it could be cleaved from the solid support by treatment with aqueous sodium hydroxide at pH 12-14 (**52.3** → **52.4**) and purified by HPLC.

Scheme 52[a]



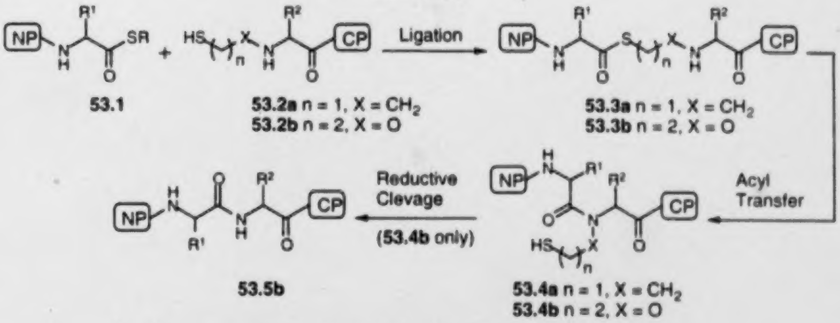
[a] NP = *N*-terminal peptide; CP = *C*-terminal peptide; ○ = cellulose resin

Solid Phase Chemical Ligation in the *C* to *N* direction has been applied to the synthesis of a 27-residue model peptide using two iterations of the coupling sequence, as well as to the synthesis of a 118-residue protein using three iterations of the sequence.[77]

1.5.2 Ligation by Thioester Exchange Involving Derivatized Glycine or Alanine

The second approach to peptide segment coupling by *Native Chemical Ligation* avoids the strict requirement for an *N*-terminal cysteine on the *C*-terminal peptide segment, and the scope of the process was expanded to permit coupling between either glycine or alanine in one segment and some other amino acid in the other segment. This version[75] of *Native Chemical Ligation* is outlined in Scheme 53. As in the cysteine-based approach, ligation occurs by nucleophilic attack by the thiol component (**53.2a**,**b**) on a thioester (**53.1**). The thiol component in this case is a glycine or alanine residue that is derivatized as either an *N*-ethanethiol (**53.2a**) or an *N*-(oxyethane)thiol (**53.2b**). These derivatized

Scheme 53[a]

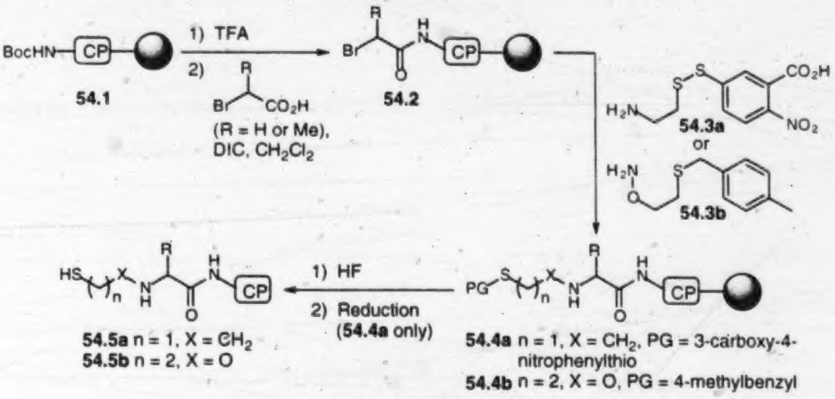


[a]R = Bn, or 5-thio-2-nitrobenzoic acid; R^1 = amino acid side-chain; R^2 = H, or Me; NP = *N*-terminal peptide; CP = *C*-terminal peptide

residues constitute the *N*-terminal amino acids of the corresponding *C*-terminal peptide chains. The product of the ligation (**53.3a** or **53.3b**) is a new thioester in which the thioacyl and α-amino groups are close to one another. Thus, a proximity induced *S*→*N* acyl transfer takes place to give the coupled peptide (**53.4a,b**). In the case of **53.4b**, the linking element used to correctly position the acyl and amino groups is then removed by reductive cleavage (**53.4b** → **53.5b**). However, for compound **53.4a**, no simple method for removing the linking element is available, and so a coupled product containing a non-standard amino acid is obtained.

Synthesis of the thioester-derivatized peptides **53.1** was achieved using the same approach as for the cysteine-based coupling strategy described above (*cf.* Scheme 45). However, the *C*-terminal peptides in the present case were made using a special approach that is summarized in Scheme 54. Standard solid phase peptide synthesis was used to generate the resin-bound peptide **54.1**. This was deprotected at its amino terminus, and then coupled either with bromoacetic acid (**54.1** → **54.2**, R = H) or with (±)-2-bromopropanoic acid (**54.1** → **54.2**, R = Me). The bromide so obtained was then displaced using either amine **54.3a** or **54.3b** to obtain compounds **54.4a** and **54.4b**, respectively. Treatment of **54.4b** with HF then served both to deprotect the peptide and

Scheme 54[a]



[a]CP = *C*-terminal peptide

cleave it from the solid support (**54.4b** → **54.5b**). In the case of **54.4a** the same process was applied, but a final reductive deprotection step had to be performed in order to liberate the terminal thiol, which had been protected as a disulfide (**54.4a** → **54.5a**). In the case where (±)-2-bromopropanoic acid was used to generate **54.2**, a mixture of peptides, epimeric at the *N*-terminal alanine reside, was obtained.[81]

In order to test the efficiency of the coupling process outlined in Scheme 53, several model studies were undertaken. The peptides used for this purpose are shown in Table 9, and the results of the coupling reactions are collected in Table 10. Ligations 1 and 2 (see Table 10) established that straightforward coupling could be achieved using either the *N*-ethanethiol (**53.2a**) or the *N*-oxyethanethiol (**53.2b**) derivative. The reactions proceeded

Table 9[75]

Model Peptides Used to Study the Approach of Scheme 53

Model Ligation	*N*-Terminal Peptide (**53.1**)	*C*-Terminal Peptide (**53.2**)
1	**53.1** R = Tnb,[a] R^1 = H, NP = LYRA	**53.2a** R^2 = H, CP = AGPAGD-NH_2
2	**53.1** R = Tnb, R^1 = H, NP = LYRA	**53.2b** R^2 = H, CP = RNTATIMMQRGNFR-NH_2
3	**53.1** R = Bn, R^1 = Bn, NP = LYRA	**53.2b** R^2 = H, CP = RNTATIMMQRGNFR-NH_2
4	**53.1** R = Bn, R^1 = H, NP = LYRA	**53.2b** R^2 = Me, CP = ARHTVHQRHLHG
5	**53.1** R = Bn, R^1 = Bn, NP = LYRA	**53.2b** R^2 = Me, CP = ARHTVHQRHLHG

[a]Tnb = 5-thio-2-nitrobenzoic acid

in good yield and within a reasonable time. The third model ligation provided an example of coupling of a more sterically hindered system and, compared to ligations 1 and 2, the rate of coupling was noticeably slower and gave poorer yields. A similar rate and yield decrease was seen for the fourth model ligation which, like the third, proceeded through a more sterically hindered transition state than either of the first two examples. Ligation 5 involved a coupling in which both the amino acids to be linked were substituted at their respective α-carbons. In this case the degree of steric hindrance was too severe and no coupling product was observed.

Table 10[75]

Results of Model Study for the Approach of Scheme 53

				Approximate Yield (%)	
Model Ligation	pH	Time (h)	Temp (°C)	Unrearranged Product (**53.3**)	Rearranged Product (**53.4**)
1	7.0	4	25	not detected	90
2	7.5	16[a]	25	not detected	75
3	7.5 then	11.5	37	30	35
	4.5	10		0	64
4	7.5 then	17.5	37	39	52
	4.5	6.5		20	69
5	7.5 then	19	37	58	not detected
	4.5	22		52	not detected

[a] >80% complete after 1 h; extended reaction time was to insure complete acyl transfer

Notable features of the data in Tables 9 and 10 are the low rates of acyl transfer with sterically hindered systems, compared to the cysteine-based *Native Chemical Ligation Strategy* described earlier. In fact, the acyl transfer rates were so low that in each of the final three model ligations the unrearranged product was stable enough to be isolated. Thus, for practical purposes, the present method would appear to be limited to glycine at the ligation site; in the case of alanine, it has not yet been established that the key displacement (**54.2** → **54.4a,b**) can be done in a stereocontrolled way.

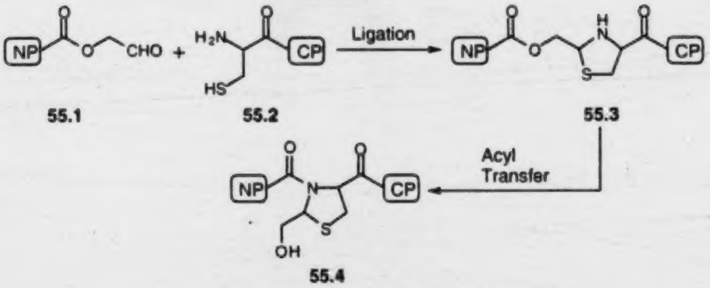
1.6 *Orthogonal Ligation Coupling Strategies*

Several methods for coupling peptide segments — collectively referred to as *Orthogonal Ligation Coupling Strategies* — have been developed by Tam. Each of these methods is characterized by the type of reaction used to ligate the peptide segments prior to the peptide bond-forming acyl transfer reaction and, in all but two examples, the site of segment coupling occurs at a cysteine, a modified cysteine, or at a residue that becomes a cysteine in the final product, as described below.

1.6.1 *Ligation by Thiazolidine Formation*

The first approach developed by Tam is one in which ligation of the peptide segments occurs by generation of a thiazolidine, and the general principle is outlined in Scheme 55.[23,82] The *C*-terminus of the *N*-terminal peptide segment is derivatized as a glycoaldehyde (**55.1**). The aldehyde group condenses with a cysteine residue located at the *N*-terminus of the other segment (**55.2**). The condensation is highly selective and gives the acyl transfer intermediate containing a thiazolidine (**55.1** + **55.2** → **55.3**). Acyl transfer proceeds through a five-membered transition state from this species to give the rearranged system **55.4**, which possesses a new peptide bond. However, unlike most of the segment coupling approaches described so far, the new peptide bond is not a part of a native peptide backbone because the chain incorporates a thiazolidine at the site of coupling.

Scheme 55[a]



[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

(a) Mechanistic and Model Studies

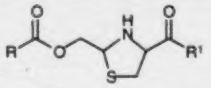
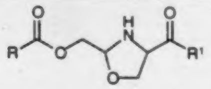
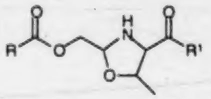
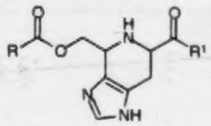
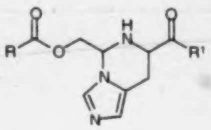
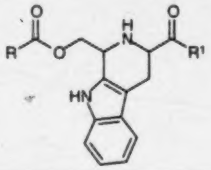
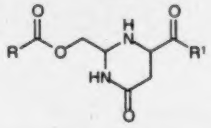
Ligation of the peptide segments by thiazolidine formation occurs in a highly specific manner even though unprotected peptides are used. This results from the fact that, of the potentially many different ligation products that could form from nucleophilic attack on the aldehyde, only the thiazolidine (*cf.* **55.3**) is sufficiently stable in the aqueous environment in which the coupling is done and so is present in a significant amount. Reactions leading to other ligation products, such as imines or acetals, would be readily reversible under the conditions used, and none of these species would be expected to be present in a significant concentration.

The thiazolidine unit can be formed only by reaction between the aldehyde group of the glycoaldehyde-derivatized peptide (*cf.* **55.1**) and the amino and thiol groups of a cysteine residue at the *N*-terminus of the other peptide (*cf.* **55.2**). This high specificity requires that the *N*-terminus of the glycoaldehyde-derivatized peptide should be incapable of forming a stable heterocycle (*cf.* Table 11) by reacting with the glycoaldehyde unit. When this requirement is satisfied, no residue other than the *N*-terminal cysteine of the *C*-terminal peptide would have two nucleophilic groups suitably disposed to allow formation of a stable heterocycle.

The possibility of effecting ligation using an *N*-terminal residue other than cysteine, but still having a nucleophilic side-chain, has also been explored.[83] A stable heterocyclic ligation product can be envisioned when either threonine, serine, tryptophan, histidine, or asparagine is the *N*-terminus of the *C*-terminal peptide (see Table 11), and a special technique[83] was developed to establish whether or not such heterocycles do actually form under ligation conditions. The experiments revealed that each of the expected heterocycles was indeed produced, but that cysteine was the most effective in terms of rapidity and completeness of reaction. Next to cysteine was threonine, which did react completely, but at a significantly lower rate. Tryptophan, histidine, and serine were found to react even more slowly than threonine, and only the reaction involving tryptophan went to

Table 11[83]

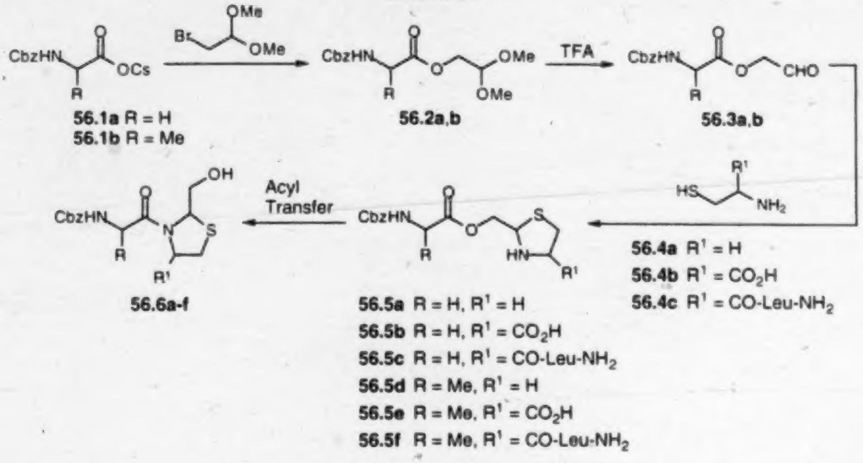
Heterocyclic Ligation Products Resulting from Reaction Between Glycoaldehyde-Derivatized Peptides and Various *N*-Terminal Residues

N-Terminus of *C*-Terminal Peptide	Ligation Product	*N*-Terminus of *C*-Terminal Peptide	Ligation Product
Cysteine		Serine	
Threonine		Histidine	 or 
Tryptophan		Asparagine	

completion. When asparagine was used as the *N*-terminal residue, almost no reaction was observed.

Initial model studies[23,82] (Scheme 56) for the thiazolidine-based ligation involved reacting a glycoaldehyde derivative of glycine or alanine (**56.3a**,**b**) with certain 1,2-aminothiols (**56.4a**-**c**). In order to generate the required glycoaldehyde derivatives, the cesium salts **56.1a**,**b** of the corresponding N^{α}-Cbz-protected amino acids were treated with bromoacetaldehyde dimethyl acetal, to give compounds **56.2a**,**b**, from which aldehydes **56.3a**,**b** were obtained by the action of TFA. At pHs between 5 and 6, the ligation products **56.5a**-**f** were obtained within approximately 15 min.[23] The reaction could be carried out at a pH as low as 2, but in such cases several hours were required for completion.[82] At either neutral or basic pH, ligation was complete in less than 5 min,[23] but competing ester hydrolysis was observed at basic pH. It was desirable to carry out the ligation at acidic pH (generally pH 4-5[82]), not only to avoid ester hydrolysis, but also to avoid complicating reactions with other nucleophiles, such as those on the side-chains of lysine and arginine.[23,82,83]

Scheme 56



In general, once the ligation product had formed, the acyl transfer (**56.5a-f** → **56.6a-f**) could be initiated by increasing the pH of the solution.[23] Half-times for the acyl transfer with three different systems were obtained at various pHs (see Table 12). From these data, it can be seen that for the 2-aminoethanethiol derivatives **56.5a,d** an increase in pH gave a corresponding increase in the acyl transfer rate. In the case of the acyl transfer intermediate **56.5e**, however, this trend was not observed, and the highest rate occurred at approximately pH 7. Likewise, compounds **56.5c** and **56.5f** were found to undergo acyl transfer most readily[23,82] at the relatively low pH of 4-5 (data not shown in Table 12), but the reactions were slow (half-time *ca.* 20 h[23] and 24 h,[82] respectively).

Table 12[23]

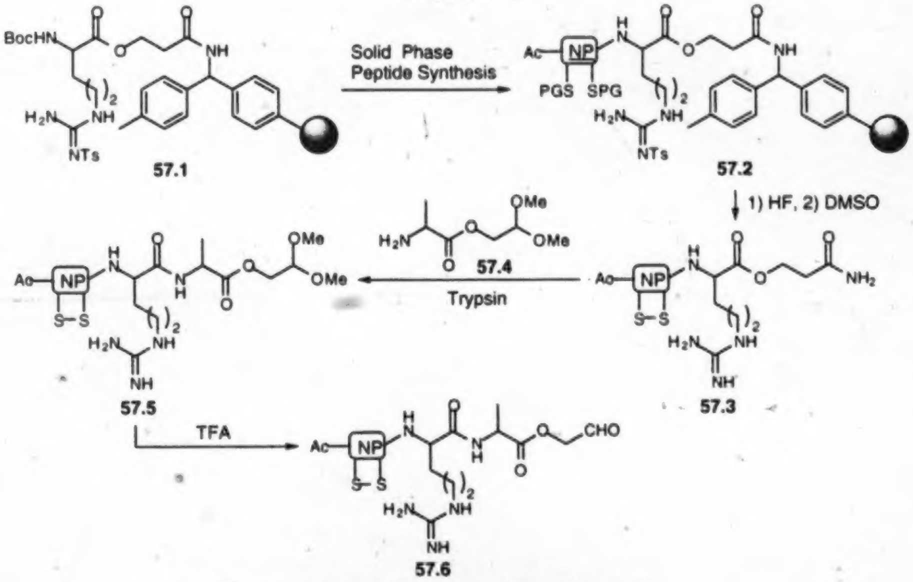
Half-Times for Acyl Transfer of **56.5a,d,e** at Various pHs

	Acyl Transfer Half-Time (h)				
Substrate	pH 6	pH 7	pH 7.4	pH 8	pH 9
56.5a	21.5	8.8	-	3.4	2.8
56.5d	37.5	22.2	20.2	9.9	3.4
56.5e	55.0	8.4	9.5	11.0	9.3

(b) Synthesis of Peptide Segments

In order to apply the thiazolidine-based approach in a practical way, an effective means of attaching the glycoaldehyde moiety to the carboxyl terminus of the *N*-terminal peptide had to be found, and two methods were devised. In the first,[23,82] an enzymatic technique was used to couple a glycoaldehyde-derivatized amino acid to a peptide segment that was protected only at its *N*-terminus and at cysteine side-chains, and had its carboxyl terminus in the form of an ester. The synthesis[82] of this particular segment was based on solid phase techniques starting from a support such as **57.1**[84] (Scheme 57), which allows the required peptide segment to be liberated from the solid support with its carboxyl terminus already in the form of an ester — in the case of **57.1**, in the form of a 3-propylamido ester. Once the required synthetic peptide had been built up (**57.1** → **57.2**), side-chain deprotection and resin cleavage could be effected by treatment with HF, and this step was followed by oxidation of the cysteine residues to the corresponding disulfides

Scheme 57[a]

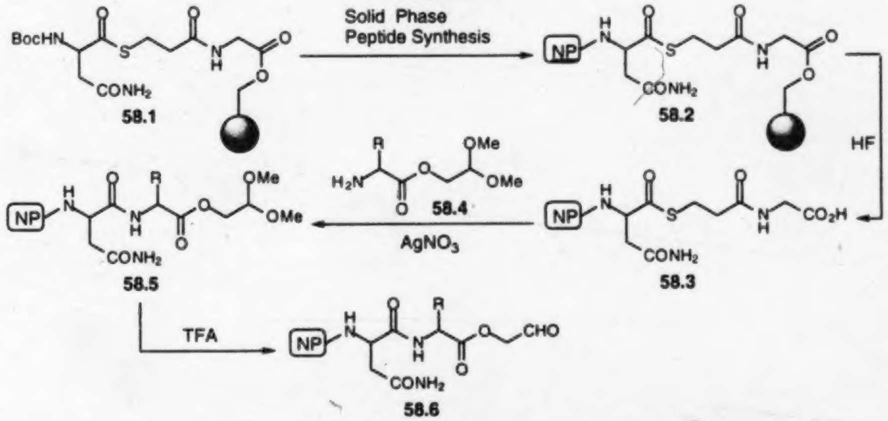


[a]PG = 4-methylbenzyl; NP = protected *N*-terminal peptide; NP = unprotected *N*-terminal peptide; ● = polystyrene resin

(**57.2** → **57.3**). At this point, the minimally protected ester was subjected to trypsin-catalyzed coupling to the dimethoxyethyl ester of alanine (**57.3** + **57.4** → **57.5**). The species obtained from this reaction was then treated with TFA to give the required glycoaldehyde-derivatized peptide (**57.5** → **57.6**).

The second approach to glycoaldehyde-derivatized peptides relied on purely chemical means,[85] thereby avoiding possible limitations imposed by substrate specificity of enzymatic reactions. The *N*-terminal peptide was synthesized by a solid phase procedure, as before, but using the thioester-containing support **58.1** (Scheme 58), with the asparagine residue being required as part of the target peptide.[85] Once the required peptide had been obtained (**58.1** → **58.2**), treatment with HF served both to deprotect the side-chains and cleave the resin (**58.2** → **58.3**). The unprotected thioester-derivatized peptide was treated with a large excess of an appropriately derivatized amino acid (**58.4**) in the presence of silver ion, to give the masked form of the required glycoaldehyde-containing peptide (**58.5**).[85] Under these conditions, acylation of nucleophilic side-chains was not observed. As in the previous method, treatment with TFA effectively removed the protecting group, liberating the free aldehyde (**58.5** → **58.6**).

Scheme 58[a]

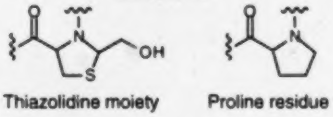


[a]NP = protected *N*-terminal peptide; NP = unprotected *N*-terminal peptide; ● = PAM resin

(c) Application to Peptide Synthesis

The thiazolidine-based *Orthogonal Ligation Coupling Strategy* has been applied to the synthesis of a 15-residue peptide,[23] a 50-residue peptide,[82] and two HIV-1 protease analogs, each 99 residues in length.[85] As mentioned previously, the coupling products obtained from these reactions do not possess a native peptide backbone, as a thiazolidine moiety is incorporated into the backbone at the site of coupling. This moiety bears a strong structural resemblance to a proline residue (see Scheme 59) and, therefore, could potentially serve as a surrogate for proline.[86] It was this observation that suggested the synthesis of the HIV-1 protease analogs, since assessment of their biological activity could provide a means of testing how well the thiazolidine unit can serve in place of proline.

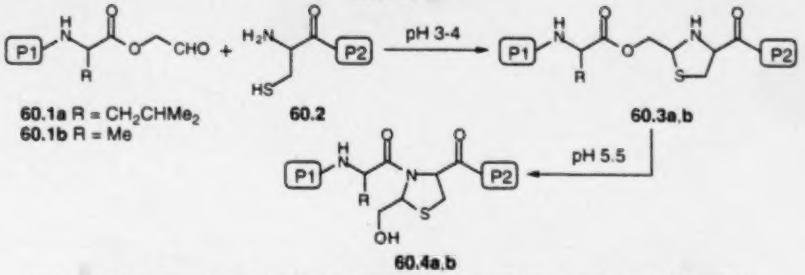
Scheme 59



HIV-1 protease contains several proline residues, two of which are suitably positioned to serve as the coupling site. The one that was ultimately chosen for the coupling reactions was equivalent to proline-39 of the native protein. Consequently, each of the two analogs **60.4a,b** (Scheme 60) had a thiazolidine unit substituted for proline-39. In addition, cysteine-67 and cysteine-95 were replaced by α-aminobutyric acid. These substitutions were made in order to avoid potentially complicating reactions and, since the residues were known not to be involved in disulfide formation in the native protein, it was assumed that their replacement would not profoundly effect enzymatic activity. In one of the analogs (**60.4b**), leucine-38 of the native protein was substituted by alanine.

The *C*-terminal peptides required for assembly of the analogs corresponded to residues 40-99 of the native peptide, with an additional cysteine at their *N*-termini. These peptides (with the indicated substitutions) were synthesized using conventional solid phase

Scheme 60^a



^{a}P1 = PQITLWQRPLVTIRIGGQLKEALLDTGADDTVLEEMN; P2 = GKWKPKMIGGIGGFIKVRQYDQIPVEIαGHKAIGTVLVGPTPVNIIGRNLLTQIGαTLNF; α = α-aminobutyric acid

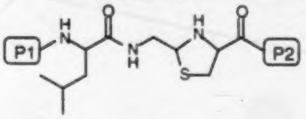
methods and were used in a fully deprotected form.

The *N*-terminal peptide segments used for the synthesis of the two HIV-1 protease analogs were each 38 residues in length and had their carboxyl termini in the required glycoaldehyde ester form, but were otherwise unprotected. These peptide segments were generated using the non-enzymatic approach (see Scheme 58).

In each case the ligation was initiated by combining the amino component (**60.2**) and the acyl component (**60.1a,b**) in a mixture of acetonitrile and water at pH 3-4.[85] Under these conditions ligation (**60.1a,b** + **60.2** → **60.3a,b**) was approximately 60-80% complete within 5-10 h. Although faster ligation would presumably have occurred at a higher pH, an increase in the pH beyond 4 resulted in precipitation of the peptide segments from solution.[85] The initial ligation had to be carried out in the presence of an aspartic protease inhibitor in order to prevent enzymatic cleavage (i.e. self-destruction) of the peptides. Once ligation was complete, the products (**60.3a,b**) were isolated, purified by HPLC, and then dissolved in a denaturing solution of guanidine hydrochloride and glycerol at pH 5.5, in order to effect acyl transfer (**60.3a,b** → **60.4a,b**). After 3-4 days of incubation, the coupled products were obtained in greater than 90% yield.

The enzymatic activity (at 22 °C) of the two synthetic analogs (**60.4a,b**) was compared to that of both the native protein and a third analog (**61.1**, Scheme 61), which

Scheme 61[a]



61.1

[a]P1 = PQITLWQRPLVTIRIGGQLKEALLDTGADDTVLEEMN; P2 = GKWKPKMIGGIGGFI-KVRQYDQIPVEIαGHKAIGTVLVGPTPVNIIGRNLLTQIGαTLNF; α = α-aminobutyric acid

represented a stable form of the acyl transfer intermediates leading to **60.4a,b**. The kinetic data, which are listed in Table 13, show that each of the synthetic peptides has a binding affinity (K_m) similar to that of the native enzyme, but each peptide has a different catalytic activity. The analog with only the proline and cysteine substitutions (**60.4a**) retained essentially complete activity, compared to the native enzyme, and the one with the additional leucine substitution (**60.4b**) showed approximately 70% of the activity. On the other hand, the analog representing the non-rearranged acyl transfer system (**61.1**), had only 30% of the activity of the wild type enzyme.

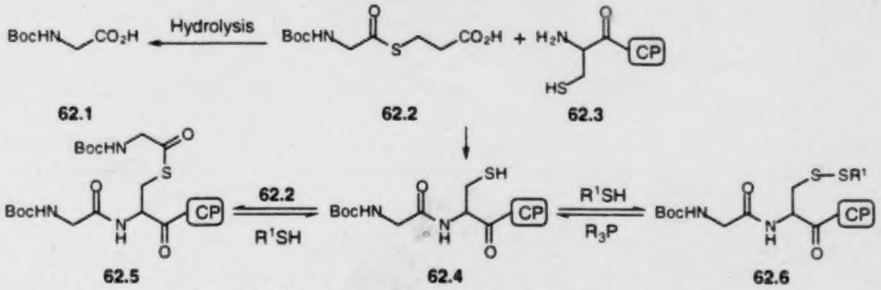
Table 13[85]
Kinetic Data of Synthetic HIV-1
Protease Analogs Compared to Wild Type

Substrate	K_m (μM)	V_{max} (μmol/min·mg)	V_{max}/K_m (μmol/min·mg·M)
Wild Type	10.1	3.43	3.40×10^5
60.4a	11.9	3.96	3.33×10^5
60.4b	8.2	2.26	2.76×10^5
61.1	11.4	1.14	1.00×10^5

1.6.2 Ligation by Thioester Exchange

The second approach used by Tam to couple peptide segments is closely related to the cysteine-based *Native Chemical Ligation Strategy* discussed above (*cf.* Scheme 41), but the thioester leaving group (SR in Scheme 41) in the present case is derived from 3-mercaptopropanoic acid.[87] Using a thioester of this type (*cf.* **62.2**, Scheme 62), an extensive investigation was carried out in order to determine optimal conditions for

Scheme 62[a]



[a]$R^1 = CH_2CH_2CO_2H$ or **62.3**; $R_3P = (HO_2CCH_2CH_2)_3P$; CP = FKA

coupling two peptide segments. The model system used for this study consisted of peptide **62.3** and the *N*-protected glycine thioester **62.2**. These components were allowed to react in solution at various pHs and, in each case, four different main products were identified (**62.1**, **62.4**, **62.5**, and **62.6**, Scheme 62). The relative amounts at each pH are shown in Table 14. The observed decrease in hydrolysis of **62.2** with increasing pH is consistent with mechanisms in which, for this pH window (5.6-7.6), each of the following conditions is met: (i) the hydrolysis shows no dependence on pH, (ii) the pK_a of the cysteine thiol lies above the highest pH studied, and (iii) the thiolate anion acts as the nucleophile.

Table 14[87]
Product Distribution for
Reaction Between 62.2 and 62.3

	Product Yield (%)			
pH	62.1	62.4	62.5	62.6
5.6	78	5	2.5	12
6.6	34	43	3	20
7.2	38	44	3	16
7.6	15	56	4	25

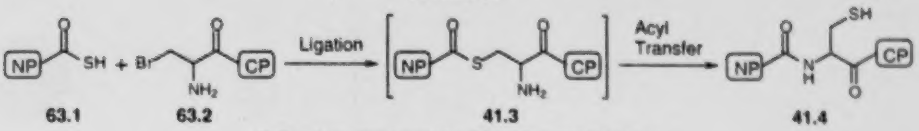
Further attempts were made to optimize the coupling under the basic conditions that minimize thioester hydrolysis.[87] This was achieved through an examination of the effect that a reducing environment would have on the product distribution for the reaction between **62.2** and **62.3** at pH 7.2. The reducing agents examined were 3-mercaptopropanoic acid and tris(2-carboxyethyl)phosphine. These were added in various amounts either alone or in combination, and it was found that almost exclusive formation of the desired product (**62.4**) could be achieved in the presence of a 2:10 mixture of tris(2-carboxyethyl)phosphine and 3-mercaptopropanoic acid. Under these conditions, formation of byproducts was almost completely suppressed. These optimized conditions were used[87] to synthesize several peptides ranging in size from 9-54 amino acids and, in each case, the coupling reactions proceed effectively, giving the acyl transfer products in yields of 60-88%.

Tam has also explored segment coupling based on ligation by thioester exchange with a homocysteine residue[88] that takes the place of cysteine at the *N*-terminus of the *C*-terminal segment in his previous examples (*cf.* **62.3**, Scheme 62). Once ligation has occurred, acyl transfer takes place, this time via a six-membered ring, and gives rise to a product that has a homocysteine residue at the site of coupling. At this point, the homocysteine residue can be methylated,[89] so that a methionine unit is now present at the coupling site. The obvious limitation of this type of segment coupling, however, is that any cysteine thiols present in either of the segments would also be methylated.

1.6.3 Ligation by Thioester Formation

The Tam group has also explored the ligation of peptide segments, prior to peptide bond formation, by reacting a thioacid and a primary bromide (Scheme 63).[87] The thioacid in this case is a derivatized form of the carboxyl terminus of the *N*-terminal peptide (**63.1**), and the primary bromide is a bromoalanine residue at the *N*-terminus of the other peptide (**63.2**). The idea underlying this approach is that, once the two components have reacted, an acyl transfer intermediate (**41.3**) identical to that obtained using either of the thioester exchange ligation procedures already described (*cf.* Schemes 41 and 62) would be obtained. In this case, however, the ligation product is not formed by thioester exchange,

Scheme 63[a]

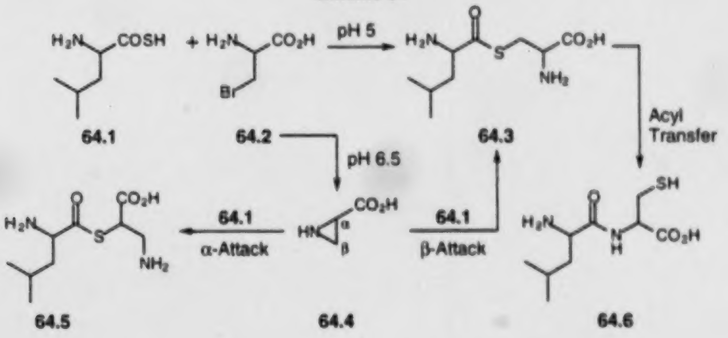


[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide

but rather by nucleophilic substitution in which the bromide is displaced by the thioacid (**63.1** + **63.2** → **41.3**). At this point, acyl transfer should proceed, as in the thioester exchange strategies, to give the rearranged, coupled product with a new peptide bond (**41.3** → **41.4**) and, just as in the thioester exchange approaches, the product will have a cysteine residue at the site of coupling corresponding to what was the *N*-terminus of the *C*-terminal peptide chain, even though cysteine was not initially present at that position.

A model study of this approach was carried out using 2-amino-4-methylpentanethioic acid (**64.1**, Scheme 64) and 3-bromoalanine (**64.2**). The reaction was studied at several pHs and the results led to the following conclusions. When the reaction was tried at a pH greater than 6, the coupling process did not proceed smoothly to the desired product, but gave instead a 6:4 mixture of the desired product (**64.6**) and compound **64.5**, respectively. Compound **64.5** was judged to result from the formation of an aziridine (**64.2** → **64.4**), followed by attack at the α-position by the thioacid (**64.4** + **64.1** → **64.5**). Attack at the β position of the aziridine would give the desired ligation

Scheme 64

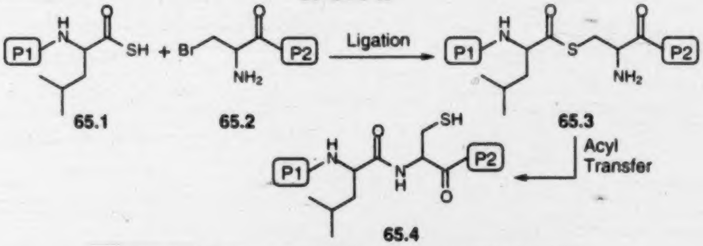


product (**64.3**) and, ultimately, **64.6**. If the pH of the solution was lowered below 5, however, it was found that very little (<3% yield) byproduct was formed. This outcome was suggested to result from the fact that at lower pH aziridine formation was not favored, and so the normal course of nucleophilic displacement of the bromide by the thioacid (**64.1** + **64.2** → **64.3**) could proceed, eventually giving the desired coupled product.

The possibility that the ligation was occurring by Michael addition of the thioacid to dehydroalanine, formed by HBr elimination from **64.2**, was excluded as follows. First, it was established that HBr elimination did not occur to an appreciable extent unless the pH of the solution was raised to about 11. Secondly, the product obtained from reaction of **64.1** and **64.2** was compared to appropriate reference samples, using HPLC. There was no evidence for epimerization, and so it was judged unlikely that ligation involved Michael addition.

The method was tested (Scheme 65) by synthesizing a 12-residue peptide (**65.4**). This was generated by coupling the fully unprotected 4-residue thioacid segment **65.1** and the bromoalanine-containing 8-residue segment **65.2**, which was protected only at an internal cysteine residue. When combined in solution, the two components reacted to give the acyl transfer intermediate **65.3**, which subsequently rearranged to the desired product in 85% yield.

Scheme 65[a]

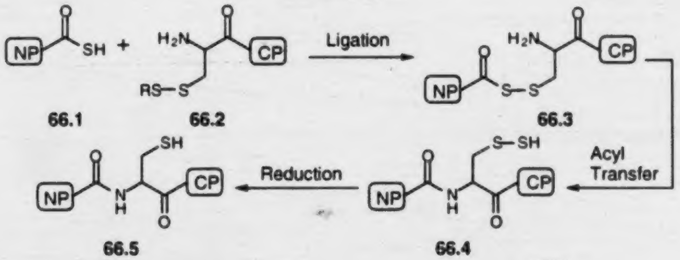


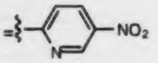
[a]P1 = SAK; P2 = PGGNAC(Acm)V; Acm = $CH_2NHCOMe$

1.6.4 Ligation by Disulfide Exchange

Another peptide segment coupling approach that the Tam group has developed is based on ligation by a disulfide bond, which is formed by attack of a thioacid on an activated thiol.[90] Like Kemp's *Prior Thiol Capture Strategy*, the activated thiol is located at the *N*-terminus of the *C*-terminal peptide (**66.2**, Scheme 66) and is part of a cysteine residue. Unlike Kemp's approach, however, the activating group is a 2-mercapto-5-nitropyridyl, rather than an Scm group. Also different from Kemp's approach is the fact that the attacking nucleophilic sulfur atom is part of a thioacid corresponding to the carboxyl terminus of the other chain (**66.1**), rather than a thiol attached to a template. In fact, no template is used in the present case; instead, the two components react in solution to form an acyl transfer intermediate *in situ* (**66.1** + **66.2** → **66.3**), and this species then undergoes rearrangement through a six-membered transition state to give the coupled product, in which the cysteine thiol is derivatized as a hydrodisulfide (**66.3** → **66.4**). This is readily reduced to generate the desired coupled product (**66.5**).

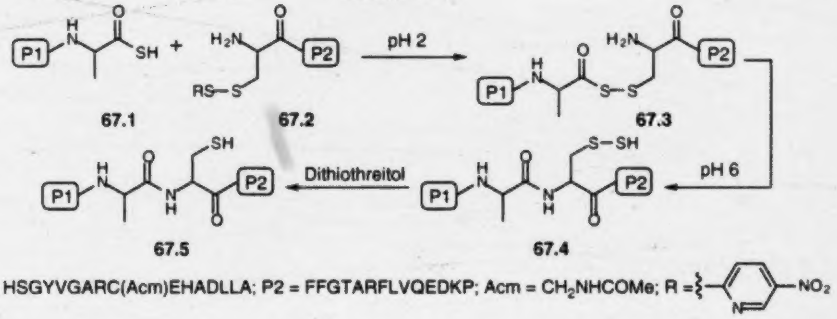
Scheme 66[a]



[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide; R =


This disulfide ligation-based route to peptide segment coupling has been applied[90] to the synthesis of a 32-residue peptide, generated by coupling a 17-residue *C*-terminal segment and a 15-residue *N*-terminal segment. The latter was made[90] by solid phase synthesis, and its *C*-terminus was in the form of a thioacid. The coupling process was initiated by activating the *N*-terminal cysteine thiol of the *C*-terminal segment, which was fully unprotected except at its internal cysteine. Activation was accomplished by treatment with 2,2'-dithiobis(5-nitropyridine). The product of this reaction (**67.2**, Scheme 67) was then combined with the thioacid-derivatized *N*-terminal peptide **67.1** in a solution of acetonitrile, water, and TFA at pH 2. Under these conditions the ligation product was formed (**67.1** + **67.2** → **67.3**). The pH was then adjusted to 6, which induced efficient acyl transfer, giving the hydrodisulfide **67.4**. Acyl transfer via the six-membered transition state accessible from **67.3** was so efficient that it was more than 90% complete after only 5 min. Finally, the hydrodisulfide **67.4** was readily reduced by treatment with dithiothreitol, to liberate the desired coupled peptide **67.5**.

Scheme 67[a]

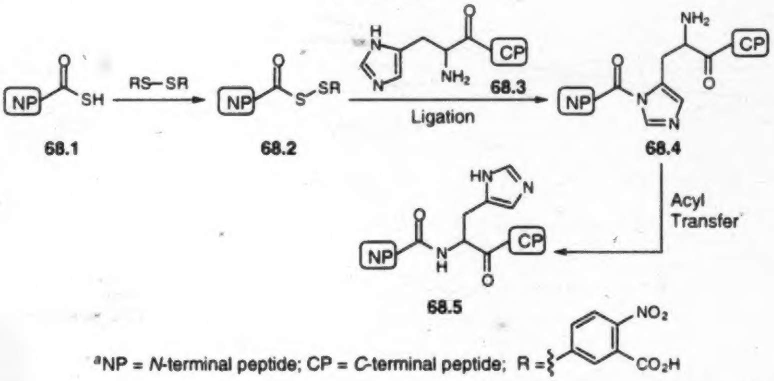


[a]P1 = HSGYVGARC(Acm)EHADLLA; P2 = FFGTARFLVQEDKP; Acm = $CH_2NHCOMe$; R =

1.6.5 Ligation by Amide Formation

One final approach to peptide segment coupling that has been reported by Tam, makes use of an *N*-terminal histidine to aid in ligation and coupling.[91] This approach (Scheme 68) provides the third example of a practical coupling technique that does not involve, either directly or indirectly, a cysteine residue. The histidine in question is positioned at the *N*-terminus of the *C*-terminal segment (**68.3**) and functions in much the same way as the terminal cysteine in the thioester exchange approaches of Kent and Tam, discussed above. In the present case, however, it is, of course, the imidazole rather than a thiol that acts as the nucleophilic ligation component. To effect ligation, the imidazole nitrogen attacks an activated thioacid moiety at the carboxyl terminus of the *N*-terminal peptide (**68.1**), and generates the transient amide **68.4**. The activated form of the thioacid is the disulfide **68.2**, which is generated *in situ* by treatment of the thioacid with 5,5'-dithiobis(2-nitrobenzoic acid). As mentioned earlier (see section 5.1a), acyl disulfides (*cf.* **68.2**) can react further to give a thioester; however, the rate of this process appears[91] to be sensitive to the reaction conditions as well as to the nature of the thioacid and, in the present case, it is believed[91] that the disulfide is actually the species involved in the ligation. Evidently, the aryl disulfide is an adequate leaving group. Once the transient amide **68.4** has formed, spontaneous intramolecular acyl transfer occurs via a six-membered transition state, and gives the coupled product **68.5**.

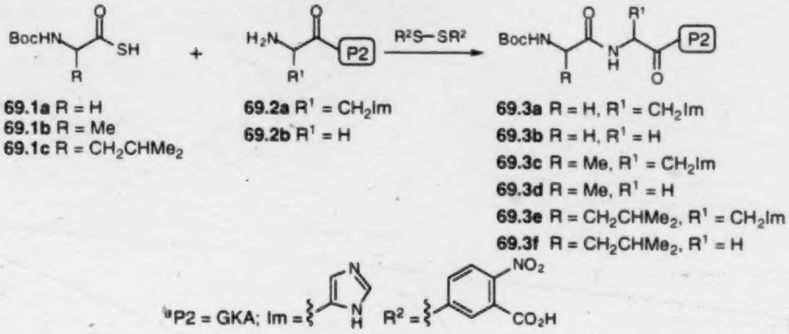
Scheme 68[a]



[a]NP = *N*-terminal peptide; CP = *C*-terminal peptide; R =

Model studies (Scheme 69) on the histidine-based coupling strategy involved the *C*-terminal peptides **69.2a** and **69.2b**, and the thioacids derived from *N*-Boc-protected glycine (**69.1a**), alanine (**69.1b**), and leucine (**69.1c**). Peptide **69.2b** provided a control that could be used for comparison to the *N*-terminal histidine peptide, in order to determine if the ligation product indeed formed prior to acyl transfer. Each of the *C*-terminal peptides used contained an unprotected lysine residue, and so the problem of

Scheme 69[a]



selectivity of acylation between the α-amino and the ε-amino groups was also examined. At pH 5, 5.7, and 6.5 each of the thioacids **69.1a-c**, once activated [with 5,5'-dithiobis(2-nitrobenzoic acid)], reacted preferentially with the α-amino group instead of the ε-amino group of the tetrapeptides **69.2a** and **69.2b**. At pH 5.7 and 6.5 both a higher selectivity of acylation in favor of the α-amino group, as well as a higher reaction rate, were observed for the peptide with the *N*-terminal histidine (**69.2a**) as compared to the one without (**69.2b**). The relative product distribution for coupling between each of the thioacids and each of the *C*-terminal tetrapeptides at pH 5.7 is shown in Table 15. As further confirmation of the role of the *N*-terminal histidine in the coupling process, a competition experiment was carried out in which an equimolar mixture of the two *C*-terminal peptides (**69.2a,b**) was treated with thioacid **69.1a** that had been activated by 5,5'-dithiobis(2-nitrobenzoic acid). In this case, the product corresponding to coupling with the histidyl

Table 15[91]

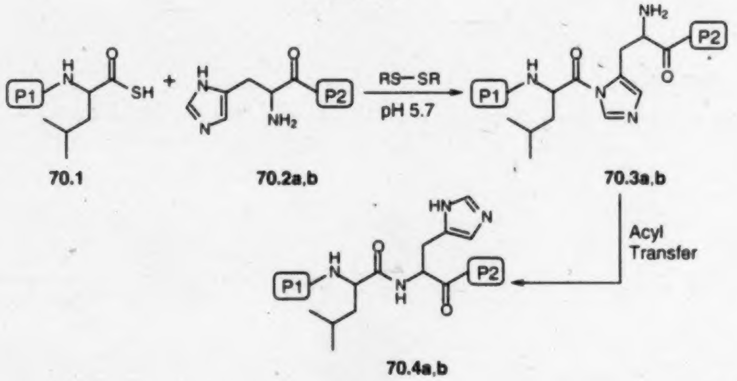
Product Distribution for Reaction of **69.1a-c** with **69.2a,b**

	C-Terminal Peptide			
	69.2a	**69.2b**	**69.2a**	**69.2b**
Thiocarboxylic Acid	Coupling Yield (%)		Acylation Selectivity (α/ε)	
69.1a	85	40	16	7
69.1b	82	50	9	5
69.1c	75	20	4	1

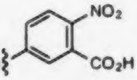
peptide **69.3a** was obtained in greater than 90% yield, whereas less than 10% of the other product (**69.3b**) was formed.

The amide ligation-based coupling procedure was further tested[91] by applying it to the synthesis of an 8-residue peptide (**70.4a**, Scheme 70) and a 25-residue peptide (**70.4b**). For each synthesis, tetrapeptide **70.1** was used as the *N*-terminal thioacid-derivatized peptide. This peptide was combined with either the 4-residue *C*-terminal peptide **70.2a**, or the 21-residue *C*-terminal peptide **70.2b**, in the presence of 5,5'-dithiobis(2-nitrobenzoic acid) at pH 5.7, to give the presumed ligation product (**70.1 + 70.2a,b → 70.3a,b**). Each acyl transfer reaction proceeded as expected under these conditions, and gave the 8-residue coupled product in 75% yield, and the 25-residue product in 60% yield.

Scheme 70[a]



[a]P1 = SKA; P2 = GKA for **a** series; P2 = LSSMERVEWLRKKLQDVHNF for **b** series; R =



2 RESULTS AND DISCUSSION

2.1 *Background*

As revealed in the introduction to this chapter, considerable effort has been invested in the development of methods for coupling peptide segments, which are based on prior ligation and proximity-induced intramolecular acyl transfer. Although this approach to segment coupling has evolved considerably since the early reports and speculations made by Brenner, Wieland, and others, the problem has by no means been solved. In fact, when we began our work in this area, we identified three main shortcomings of what was then the existing technology, and used these as the basis for our research program (see later).

2.2 Segment Coupling Approaches Involving Ligation by Thioester Exchange

At the outset of our investigations into segment coupling, we were not concerned with developing a new approach to ligating the peptide segments prior to the peptide bond-forming acyl transfer reaction, although later we did investigate and speculate about other ways of carrying out this crucial step (see Section 2.3). The ligation reaction that we chose was thioester exchange. By the time we had entered this area of research, Kent had clearly established the power of this technique for effecting ligation and we chose, therefore, to address only the weaknesses of the existing technology not directly related to the ligation step.

2.2.1 Research Objectives

(a) Development of a Generalized Approach to Peptide Segment Coupling

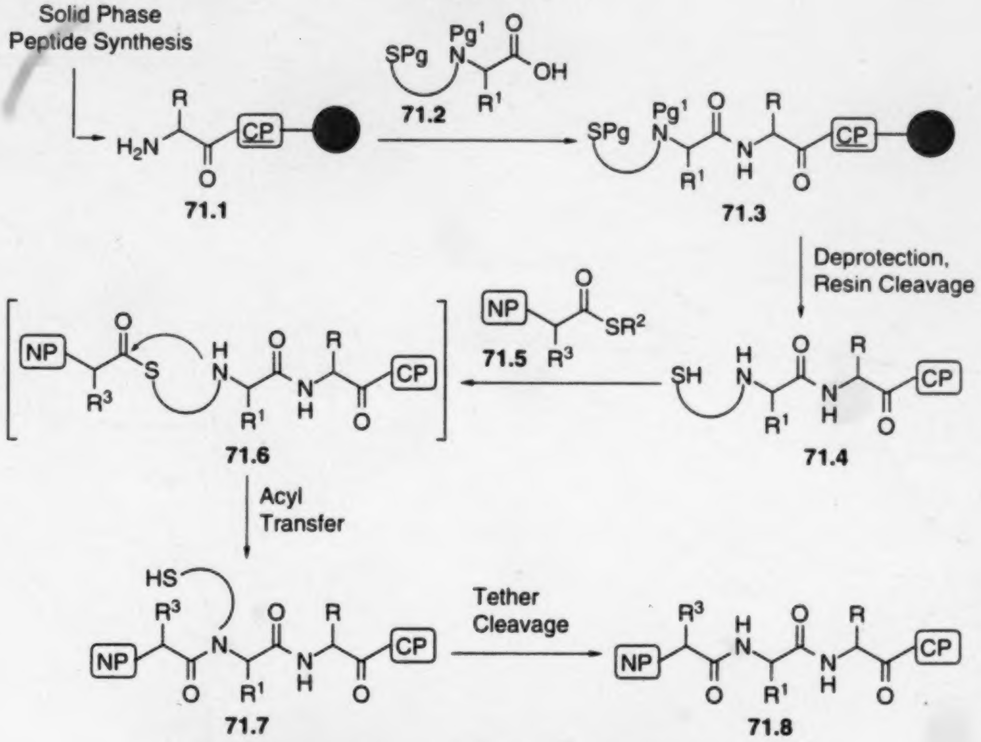
Our first and primary objective was to develop a generalized approach to segment coupling which would allow coupling to be carried out between *any* two amino acids. Kemp's early explorations into segment coupling using his *Prior Amine Capture Strategies* (see Section 1.3) were the first examples of what could potentially have become a generalized approach to segment coupling. In these strategies, initial ligation of the segments was based on the reaction of an *N*-terminal amine of what would have been the *C*-terminal peptide, with the derivatized *C*-terminal portion of the *N*-terminal segment (Scheme 3). Unfortunately, as was revealed during the course of his investigations, amine capture proved ineffective as a means of ligating peptide segments, and so the approaches were not developed further. Of the remaining approaches that have been devised for segment coupling (see Section 1), almost all involve coupling at a cysteine, a modified cysteine, or at a residue that becomes a cysteine in the final product. The few approaches that do not involve a cysteine residue are themselves limited to coupling between specific residues.

From a practical point of view, when coupling peptide segments it is likely that difficulties will result in certain cases. These difficulties may arise, for example, from having a particular sequence of amino acids at or near the coupling site, which could impose conformational or steric constraints on the system and impede the coupling process. This type of phenomenon is well-known in conventional peptide synthesis, and results in so-called "difficult sequences". It is necessary, therefore, to have available a generalized method for segment coupling, so that when difficulties do arise in joining two or more segments, modified approaches to the same peptide can be explored by joining different segments at different sites.

The plan we developed for a generalized approach is outlined in Scheme 71, and is based on the use of a specially derivatized amino acid (*cf.* **71.2**). In this approach, standard solid phase peptide synthesis techniques would be applied to generate the required *C*-terminal segment, but in the last step of the synthesis, the derivatized amino acid would be incorporated into the chain (**71.1** + **71.2** → **71.3**). Amino acid **71.2** contains a suitably protected amino group, which bears a tether that terminates in the form of a protected thiol. Both the thiol and amino protecting groups on the special amino acid would be chosen such that they could be removed in the subsequent step of global deprotection and resin cleavage (**71.3** → **71.4**).

The fully deprotected peptide (**71.4**) obtained from this process would then be used in a thioester exchange with an appropriate *N*-terminal segment (**71.5**), which would be derivatized at its *C*-terminus in the form of a thioester. This reaction would give rise to an intermediate (**71.6**) in which the required amino and carboxyl groups are in proximity

Scheme 71



[a] NP = *N*-terminal peptide; C̲P̲ = *C*-terminal peptide (protected);
CP = *C*-terminal peptide (deprotected); Pg = protecting group;
● = polystyrene resin

and, consequently, an intramolecular acyl transfer could then occur, giving rise to the rearranged compound **71.7**, which possesses a new peptide bond at the site of coupling. At this stage the tether would be removed (**71.7** → **71.8**) to give the desired coupled product.

The generality of the approach depicted in Scheme 71 arises from the ability to incorporate any specially derivatized amino acid (*cf.* **71.2**) in the final stage of solid phase synthesis of the *C*-terminal segment. One such specially derivatized residue would have to be made for each naturally-occurring amino acid, and could be made for non-naturally occurring amino acids as well. The other amino acid involved in the coupling reaction is the thioester-derivatized *C*-terminal residue of the *N*-terminal chain (*cf.* **71.5**). Again, any amino acid desired could be incorporated at this position, this time by using technology that Kent has developed for the synthesis of the *N*-terminal segments for his *Native Chemical Ligation Strategies* (see section 1.5.1c).

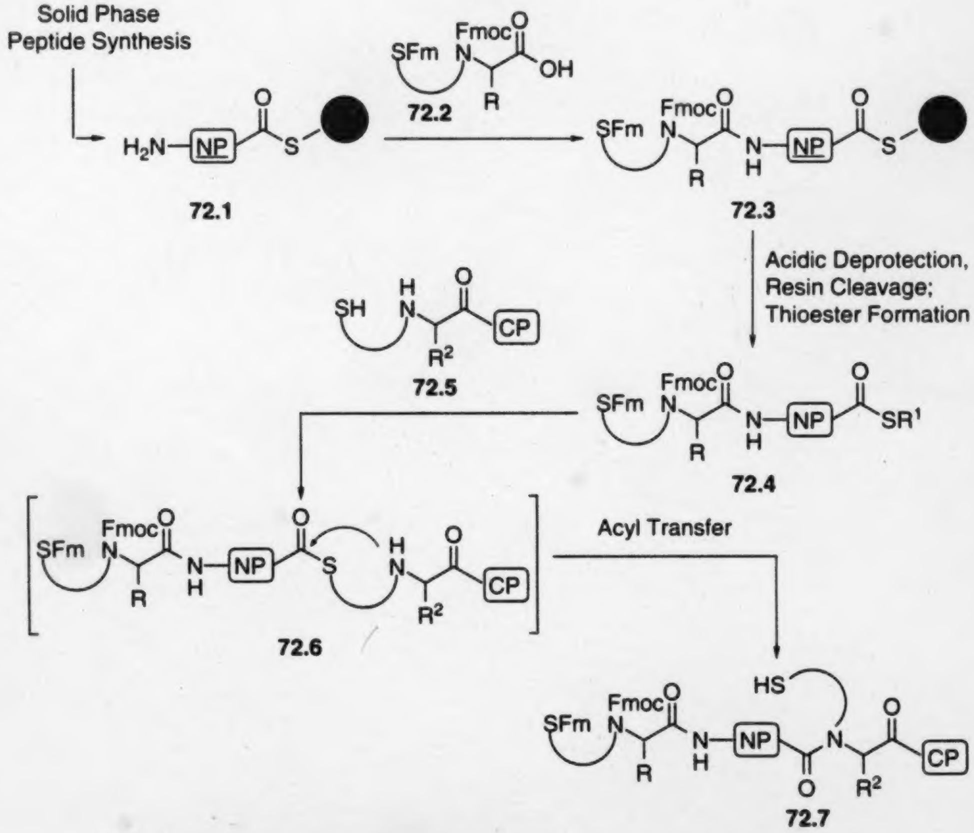
The tether used for the approach of Scheme 71 would have to fulfill certain criteria. Firstly, it would have to be constructed in such a way that, once ligation has occurred, the appropriate acyl and amino groups are in proximity so that intramolecular acyl transfer can occur. As well, the tether would need to withstand all reaction conditions met during the final stages in the preparation of the *C*-terminal segment, and during the coupling process. At the same time, the tether must be removable without damage to the newly-coupled product (*cf.* **71.7** → **71.8**). Three different, but related, tethers were examined during our work, and each will be discussed in due course (see later).

(b) Development of a Generalized Iterative Approach to Peptide Segment Coupling

The second objective of our research program was to extend the above generalized approach to segment coupling, so that it could be applied in an iterative sense. In other words, once the initial coupling reaction has been carried out between two segments (*cf.* **71.4** + **71.5** → **71.7**), the product of that reaction could be used in a subsequent coupling with a new peptide segment. When we began studying the problem of segment coupling, only one example of an iterative coupling method had been developed (see Sections 1.4.2f and g). Although the process in question did operate in the desired sense, it was somewhat cumbersome in that several steps involving protection and deprotection of variously positioned cysteine residues were required. During the course of our work, an iterative approach was reported by Kent (see Section 1.5.1e), but the technique described was not based on a generalized coupling strategy.

Extension of the generalized approach depicted in Scheme 71, so that it can be applied in an iterative way, simply requires that an orthogonal protecting group strategy be applied, as outlined in Schemes 72 and 73. Standard solid phase techniques with, for example, acid-labile protecting groups and an acid-labile thioester-based resin linker, would be used to generate the *N*-terminal peptide segment (*cf.* **72.1**). In the last step of the synthesis, a specially derivatized amino acid, having orthogonal base-labile protecting groups (in this particular example Fmoc and Fm groups are shown), would be incorporated (**72.1** + **72.2** → **72.3**). The product of this reaction would then be treated with acid in order to remove all but the terminal protecting groups and cleave the peptide from the resin, leaving it in the form of a thioacid (*cf.* Section 1.5.1c), that would subsequently be

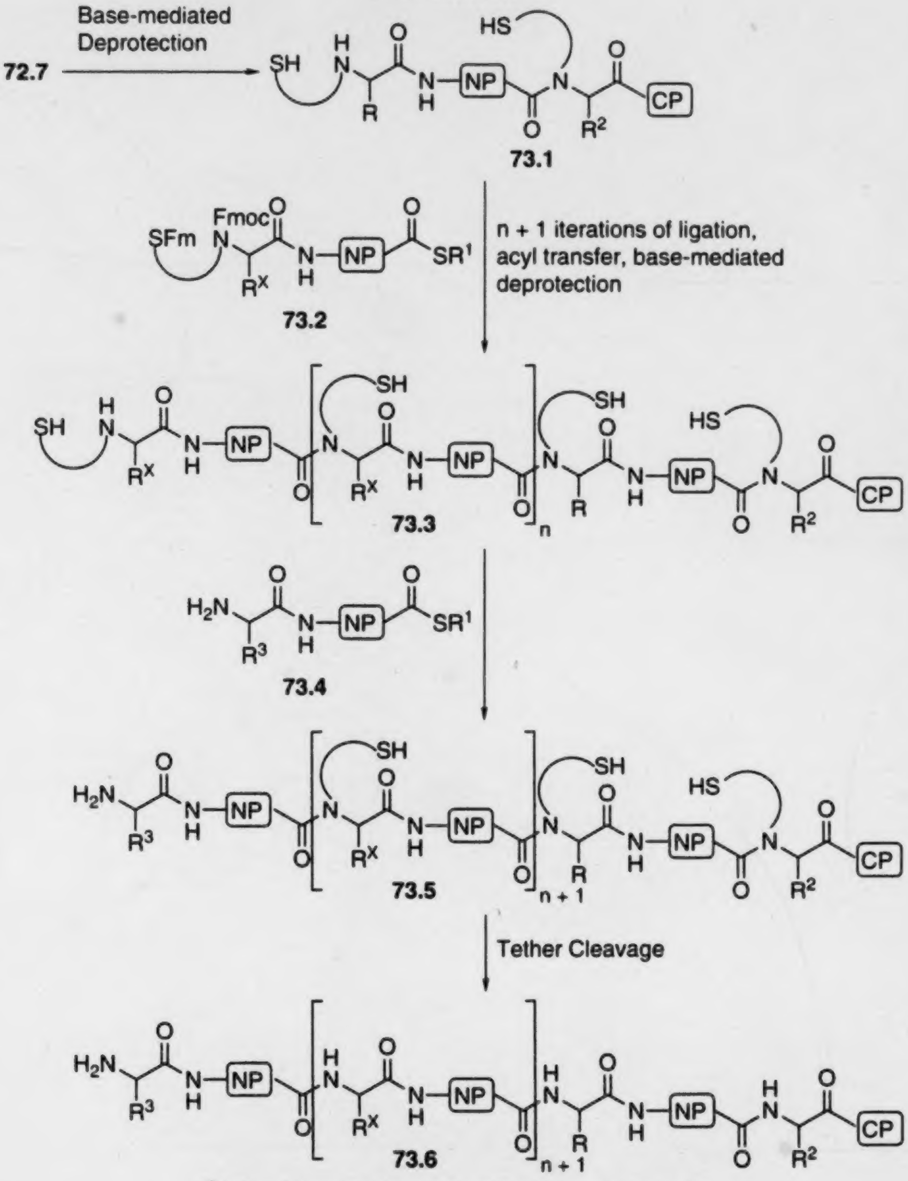
Scheme 72



[a] NP = *N*-terminal peptide (protected); NP = *N*-terminal peptide (deprotected);
CP = *C*-terminal peptide; ● = polystyrene resin;

converted into a thioester (**72.3** → **72.4**). The thioester-derivatized peptide could then be used in a ligation reaction with a *C*-terminal peptide segment (**72.5**), having an *N*-terminal

Scheme 73[a]



Base-mediated Deprotection
72.7
73.1
73.2
n + 1 iterations of ligation, acyl transfer, base-mediated deprotection
73.3
73.4
73.5
Tether Cleavage
73.6

[a] NP = *N*-terminal peptide; CP = *C*-terminal peptide

amino acid possessing the special tether described above. As before, the ligated species (**72.6**) would be expected to undergo intramolecular acyl transfer, giving the coupled product **72.7**.

At this point, the base-labile protecting groups would be removed from the *N*-terminal residue (**72.7** → **73.1**, Scheme 73), and the resulting product could then enter into another coupling cycle. In principle the process could be repeated as many times as desired (**73.1** + **73.2** → **73.3**), and then the last coupling reaction would be done with an *N*-terminal peptide (**73.4**) having at its *N*-terminus a standard residue without the tether attached (**73.3** + **73.4** → **73.5**). In the final step of the process, the tethering groups would be removed in order to liberate the desired peptide (**73.5** → **73.6**). Of course, the process could also be carried out using a base-labile resin linker and protecting groups for the synthesis of the *N*-terminal segment (*cf.* **72.4**), along with an *N*-terminal residue (*cf.* **72.2**) having acid-labile protecting groups. In such a case the product of the first coupling reaction (*cf.* **72.7**) would be activated for use in a second cycle by treatment with acid instead of base (*cf.* **72.7** → **73.1**).

(c) Adaptation to the Solid Phase

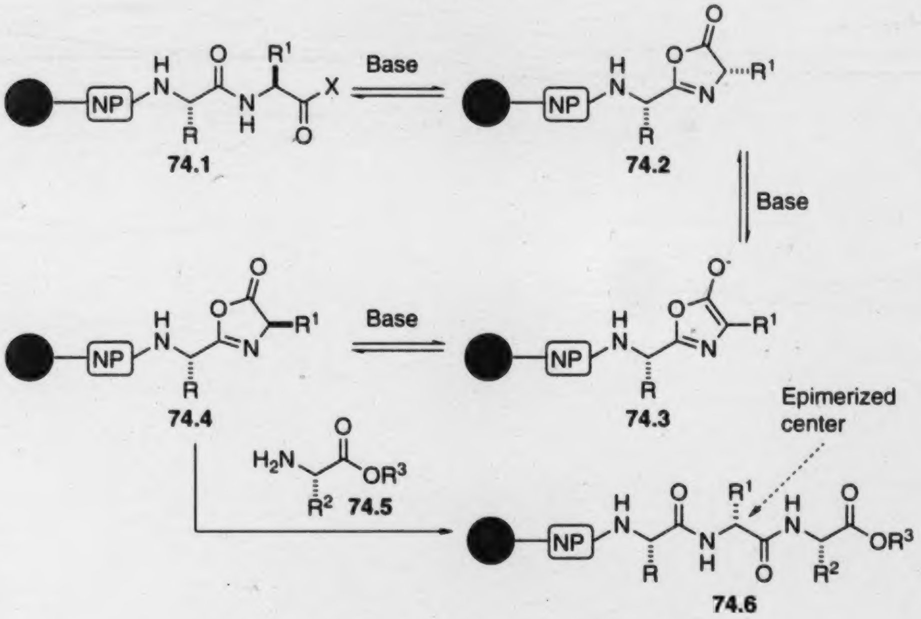
The third objective of our research was to adapt the generalized, iterative approach to the solid phase. Such an extension would have associated with it the obvious positive implications of simplifying the synthetic process, but would be particularly beneficial in the context of an iterative approach. A technique of this type could potentially lead to a system that operates much like conventional stepwise solid phase peptide synthesis, but with the benefit of coupling segments together in each cycle of the process, rather than individual amino acids, as described below. When we began work on this project no reports of a solid phase-based approach to segment coupling using a ligation strategy had been described. During our studies, however, one such report did appear (see Section 1.5.1e), but the technique described was not based on a generalized coupling strategy.

The plans we developed for adapting our proposed method for segment coupling to the solid phase require only very slight modifications. In fact, all that we would have to do is to have one or the other of the segments used in the coupling reaction attached to a resin. If the *C*-terminal peptide was to be the resin-bound peptide, then segment coupling would proceed in the *C* to *N* direction; if the *N*-terminal peptide was to be resin-bound, then synthesis would proceed in the *N* to *C* direction. In either case, one point that had to be considered was how and when the ultimate resin-bound segment would be attached to the solid support. It would not suffice, for example, to simply plan to synthesize the *C*-terminal peptide as before (*cf.* **71.1**, Scheme 71) using standard solid phase methods and, once it was derivatized with the special amino acid residue (**71.1** + **71.2** → **71.3**), to remove all the protecting groups in a way that did not cleave the product peptide from the resin. In this case, the deprotected peptide could not be used in a ligation/acyl transfer reaction, because the resin used for the stepwise portion of the synthesis would have to be one that was compatible with organic solvents, and such a resin would not be effective in the aqueous environment required for the segment coupling portion of the process, since its swelling properties would be compromised.

(i) Solid Phase Segment Coupling in the N to C direction

In standard stepwise solid phase peptide synthesis, synthesis in the *N* to *C* direction is not possible due to complications related to epimerization at the α-carbon of the individual amino acids as they are added to the growing peptide chain. This results from the propensity of the *C*-terminal residue of the growing peptide chain, once in the strongly activated form (**74.1**, Scheme 74) required for coupling with the incoming amino acid (**74.5**), to form an azlactone (**74.2**).[92] The azlactone readily undergoes epimerization via **74.3** to give the diastereomeric azlactone **74.4**, which ultimately yields the epimerized product (**74.6**). In segment coupling in the *N* to *C* direction, epimerization is not expected to be a significant side reaction. This is because the *C*-terminal residue of the *N*-terminal chain is in the form of a thioester, and it has been demonstrated (see Section 1.5.1b) that when the carboxyl terminus is in this form, it is not sufficiently activated to epimerize under standard ligation conditions (see below).

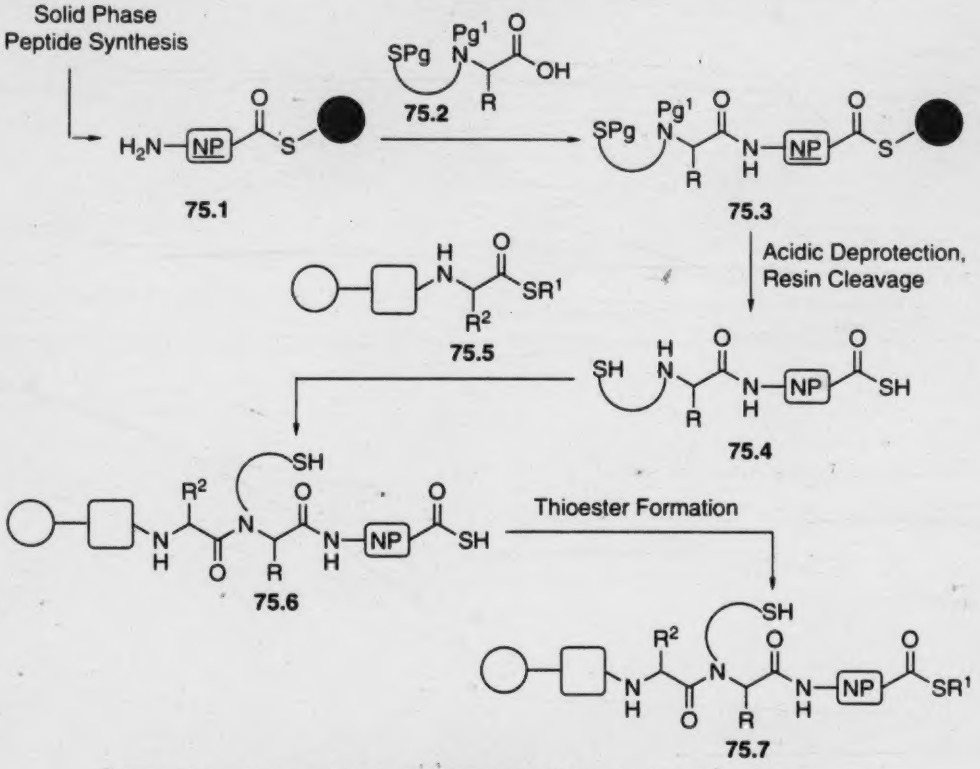
Scheme 74



^{a}NP = *N*-terminal peptide; X = activating group

Our plan for solid phase segment coupling in the *N* to *C* direction is outlined in a general way by reference to Scheme 75. The resin-linked, thioester-derivatized *N*-terminal peptide segment (**75.3**) would be generated using standard solid phase procedures, in a manner similar to what we had planned previously, but without use of an orthogonal protecting group strategy for the *N*-terminal, tether-carrying, amino acid (**75.2**). Global deprotection and resin cleavage (**75.3** → **75.4**) would be effected under appropriate conditions, giving rise to thioacid **75.4**. This would then be coupled, via ligation and intramolecular acyl transfer, to a thioester-derivitized, water-compatible resin (*cf.* **75.5**) possessing, for example, an acid-labile linker, giving **75.6**, which would subsequently be

Scheme 75

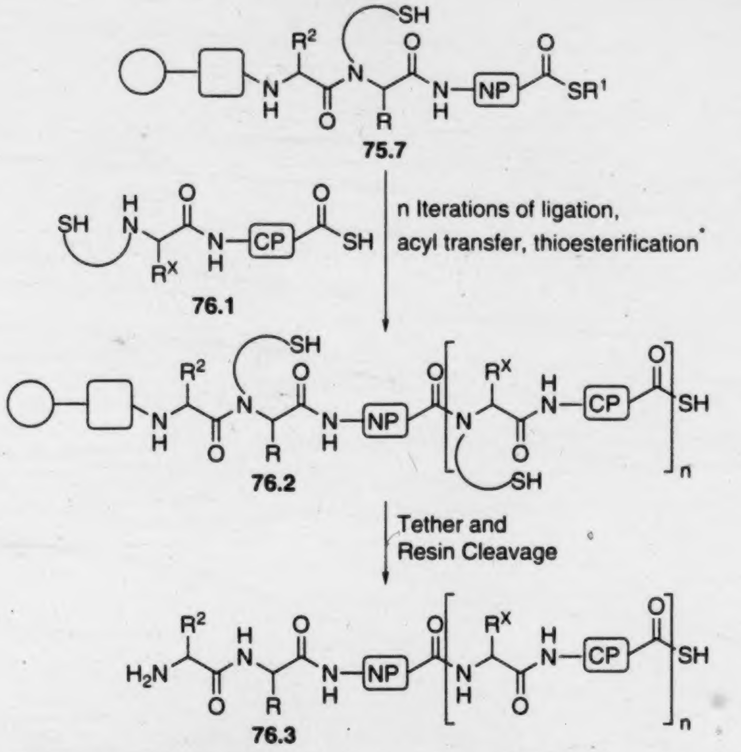


[a] NP = *N*-terminal peptide (protected); NP = *N*-terminal peptide (deprotected);
Pg = acid-labile protecting group; ● = polystyrene resin;
○ = water compatible resin; □ = acid labile linker

transformed into thioester **75.7**.

Thioester **75.7** could then be used in a subsequent ligation reaction with another appropriately derivatized peptide segment (**76.1**, Scheme 76), and this would then be followed by thioesterification. After the desired number of coupling reactions were carried out, the product (**76.2**) would then be cleaved from the resin and the tethering groups would be removed. In the present example, if the tether were acid-labile, then the single operation of treatment with a strong acid would serve both to remove the tethers, and cleave the peptide from the solid support (**76.2** → **76.3**).

Scheme 76[a]



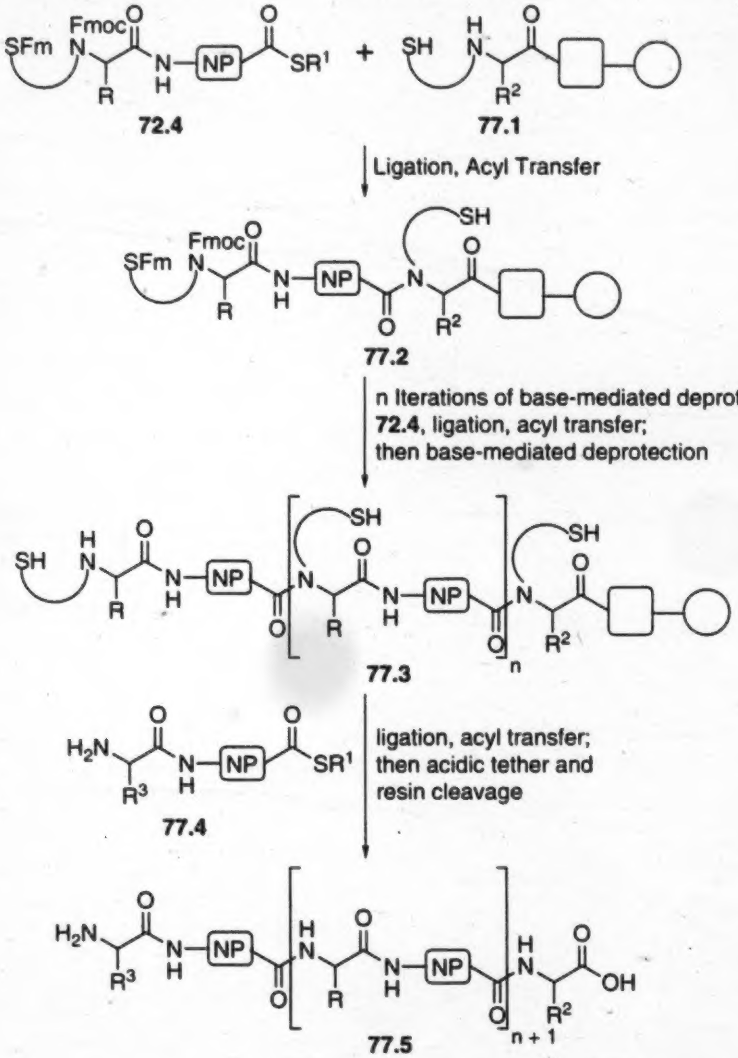
75.7
76.1
n Iterations of ligation, acyl transfer, thioesterification*
76.2
Tether and Resin Cleavage
76.3

[a] NP = *N*-terminal peptide; CP = *C*-terminal peptide;
○ = water compatible resin; □ = acid labile linker
*Thioesterification is not carried out at the end of the last coupling cycle

(ii) Solid Phase Segment Coupling in the C to N direction

Our plan for solid phase segment coupling in the *C* to *N* direction is outlined in Scheme 77. In this case, a thioester-derivatized peptide segment (**72.4**) possessing a suitably protected (Fmoc/Fm protected in the particular example shown), tether-bearing *N*-terminal residue would be prepared in the manner already described (see Scheme 72). This segment would be used in a ligation reaction with an appropriately derivatized water-compatible resin possessing an acid-labile linker (**77.1**). The product (**77.2**) of that reaction would be subjected to base-mediated deprotection of the *N*-terminal residue in order to liberate the free thioamine. This would then be treated with another suitably protected thioester-derivatized segment (*cf.* **72.4**), to give a ligated species that could then undergo intramolecular acyl transfer leading to a coupled product. The cycle would be repeated the desired number of times, and then a final treatment with base would give **77.3**. The last coupling reaction would be conducted using a *C*-terminal thioester-

Scheme 77

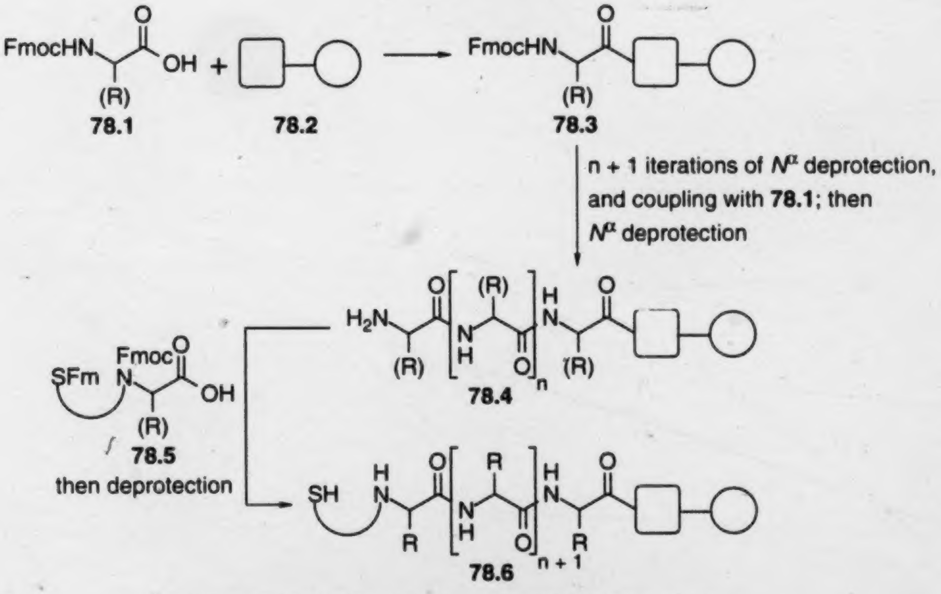


[a] NP = *N*-terminal peptide; ○ = water compatible resin; □ = acid labile linker

derivatized segment lacking the special amino acid at its *N*-terminus, and this would then be followed by treatment with strong acid which would serve to cleave the product from the resin and, if an acid-labile tether was employed, also to remove the tethering groups (**77.3** + **77.4** → **77.5**). Of course, as mentioned before, one could potentially exchange acid-labile groups for base-labile groups, and vice versa, with the appropriate corresponding modification of the conditions for cleavage.

Recent progress in the area of solid phase resin chemistry has led to the development of new resins,[93] which could potentially simplify the planned approach to solid phase segment coupling in the *C* to *N* direction just described. These resins have the unusual property of being able to maintain their swelling integrity in both organic and aqueous solvents and could, therefore, potentially be applied to prior ligation-based segment coupling in the following way (see Scheme 78). Loading of the water/organic solvent compatible resin with the first residue (**78.1**) of the segment to be synthesized

Scheme 78



n + 1 iterations of N^{α} deprotection, and coupling with **78.1**; then N^{α} deprotection

[a] (R) = protected side chain; R = deprotected side chain

○ = water/organic compatible resin; □ = acid labile linker would be done using, for example, an acid-labile linker, and would be conducted in an organic solvent (**78.1** + **78.2** → **78.3**). Standard Fmoc-based solid phase chemistry, or other suitable synthetic tactic, would then be applied in order to generate the desired segment (**78.3** → **78.4**), and then the special *N*-terminal amino acid (**78.5**) would be attached, and the resulting product would be subjected to global deprotection, removing all the protecting groups, but leaving the resin attached (**78.4** + **78.5** → **78.6**). At this point the process would essentially converge on that which is outlined above in Scheme 77 (except that a different resin is involved), with **78.6** replacing **77.1**. Of course, one could envision a complimentary approach in which acid-labile groups replaced base-labile groups, and vice versa.

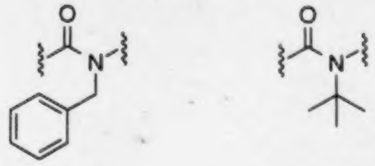
2.2.2 Considerations Regarding the Tether

(a) Tether Cleavage

The overall objectives of our research are outside the well established traditions of peptide synthesis, and in order for the methods that we planned to develop to be generally useful, we needed to maintain simplicity by achieving our objectives using, primarily, chemistry that is well-known to peptide chemists. As a result, when developing tethers we considered only those that would be cleavable by a simple operation such as treatment with acid or base.

In the rearranged coupled product (*cf.* **72.7**, Scheme 72), the nitrogen to which the tether is attached is in the form of an amide, and so the tether should cleavable by treatment with strong acid, if a relatively stable carbocation results. This would be the case, for example, if the carbon atom α to the amide nitrogen was benzylic (*cf.* **79.1**, Scheme 79) or tertiary (*cf.* **79.2**). Of course, in the case of a benzylic tether, it would also potentially be cleavable by hydrogenolysis, using a heterogeneous catalyst. However, we did not favor this approach as the presence of free thiol groups would be likely to lower catalyst activity. As well, use of heterogeneous catalysis would not be compatible with the solid

Scheme 79



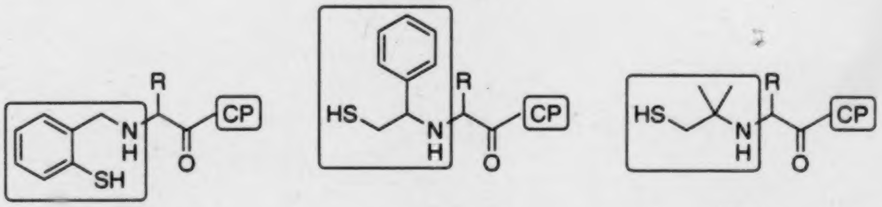
79.1 **79.2**

phase protocols that we hoped eventually to explore.

An additional matter had to be considered regarding the use of an acid-labile tether. This has to do with the fact that our proposed synthesis of the tether-bearing segment (*cf.* **72.4**, Scheme 72) includes an acid-mediated step to remove the side chain protecting groups and cleave the peptide from the resin (*cf.* **72.3** → **72.4**). Hence, our tether would have to be stable to acid prior to the amide bond-forming segment coupling and then sensitive to acid afterwards. Prior to segment coupling, the nitrogen to which the tether is attached is in the form of an amine or protected amine, and afterwards the nitrogen is part of an amide. As it turns out, it is known that a benzylic amine is stable to treatment with strong acid, whereas a benzylic amide is not.[94] We could not find any literature precedent to suggest that the same would be true for an amine attached to a tertiary carbon versus an amide, but we assumed that this would be the case.

The acid-labile tethers that we examined are shown in Scheme 80. Each of these tethers has certain features that potentially make it more or less attractive than the others for use in the ligation/acyl transfer process. For example, the two benzylic tethers could offer some degree of control with respect to their acid lability in a very straightforward way. If it turned out that the tethers are not as labile as we expect them to be — once in the form of benzylic amides — then we could simply incorporate into them a suitable electron releasing substituent, such as a methoxy group, at the *para* position, in order to stabilize the carbocation involved in tether removal. Likewise, introduction of an electron withdrawing substituent at this position should diminish the acid lability, if the benzylic tethers turn out to be too sensitive to acid. This type of control would be more difficult to achieve using the dimethyl tether. Replacement of the two methyl groups with electron withdrawing groups, such as trifluoromethyl groups, would certainly be possible if the tether was too acid-labile. However, increasing the acid sensitivity by introduction of electron releasing groups, such as silicon or boron, in place of the methyl groups, would probably be too complicated.

Scheme 80

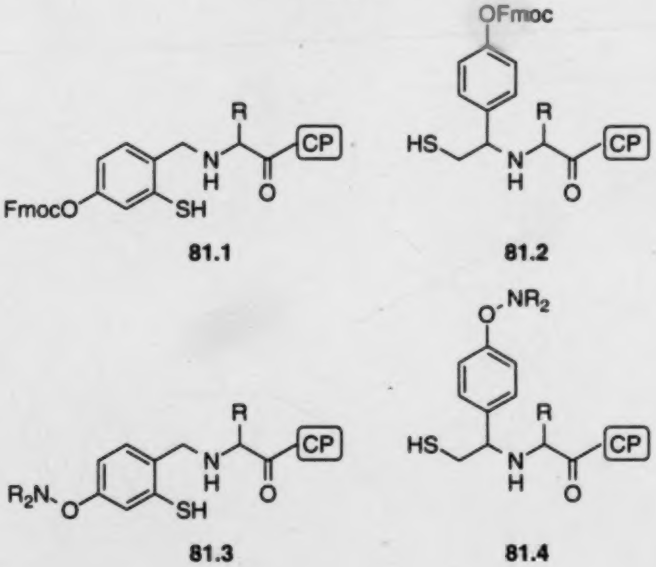


80.1 **80.2** **80.3**

Although we hoped that the tethers would behave in the desired sense — being acid-labile in the form of amides but not as amines or protected amines — we were aware of the possibility that they might be labile before the amide had formed. Of course, this would complicate matters during solid phase synthesis of the thioester-derivatized *N*-terminal segments (*cf.* **72.4**, Scheme 72), because the tether would be cleaved during the acidic deprotection and resin cleavage steps, and so would not be available for ligation and acyl transfer. The benzylic tethers that we had devised could potentially allow some control in such an event. Such control would require that an electron withdrawing group be introduced in the *para* position (so that the amine species would not be acid sensitive) that could be altered in some way following the ligation/acyl transfer reaction so that it would become electron releasing, thus causing the tether to become acid sensitive. Certain groups that fulfill this role are known, and have been applied to other problems in peptide chemistry.[95]

We also thought that it might be possible to achieve this effect by using a benzylic tether with an Fmoc protected hydroxyl group in the *para* position (*cf.* **81.1** and **81.2**, Scheme 81). The carbonate form of the hydroxyl group might be sufficiently electron withdrawing to prevent acid cleavage of the tether at the amine/protected amine stage, and could be converted to the electron releasing free hydroxyl form at an appropriate time by treatment with mild base. Another potential solution for the problem of premature acid lability would be to incorporate a hydroxylamine at the *para* position of the benzylic tethers (*cf.* **81.3** and **81.4**). During the acid deprotection step the amine would be protonated and might, therefore, prevent electron release by the adjacent hydroxyl. Following ligation and acyl transfer, the nitrogen oxygen bond could be reduced to liberate the electron releasing hydroxyl group, thereby making the tether more acid-labile.

Scheme 81



(b) *Thiol Nucleophilicity*

Aside from the cleavability of the tethers, another distinction between the three tethers depicted in Scheme 80 is the relative nucleophilicity of their respective thiol groups. Since the thiol substituent in **80.1** is aromatic, it is expected to be considerably less nucleophilic than the primary alkyl thiol groups of tethers **80.2** and **80.3**. In theory, it should be possible to increase the nucleophilic nature of the thiol group in **80.1** by placing an electron releasing group on the benzene ring in the position *para* to sulfur. However, since the thiol groups in both **80.2** and **80.3** are primary, they are likely also less sterically encumbered than the aromatic thiol. This situation implies that a very strong electron-releasing group would likely be required in **80.1**, in order to sufficiently enhance the reactivity of its sulfur atom relative to the other tethers. This could, in turn, be detrimental to the acyl transfer reaction that occurs following ligation since, in this case, a more electron rich sulfur atom would impede the acyl transfer by making the carbonyl of the thioester less electrophilic.

(c) Transition State Size and Shape

In the ligated species, the aromatic tether **80.1** would give rise to a six-membered acyl transfer transition state. Kemp has previously examined systems with a related acyl transfer transition state, but having an oxygen in place of the sulfur (see Section 1.3). For these systems, it was found that transfer was complicated due to steric interactions resulting from the compressed size of the transition state. In **80.1** the increased size of the sulfur atom could potentially alleviate such unfavorable nonbonding interactions to some extent, and so **80.1** might be more amenable to acyl transfer than those systems studied by Kemp.

The acyl transfer transition state arising from both **80.2** and **80.3** is five-membered and, consequently, should resemble that of the cysteine-based *Native Chemical Ligation* approach of Kent (see Section 1.5.1), and so is not expected to be overly problematic. However, one additional factor requires attention when considering tether **80.2**, which results from the fact that it is chiral. Since **80.2** contains a stereogenic center, two diastereomeric acyl transfer transition states will result from the ligation reaction. Obviously the matter is easily rectified by using the tether in optically pure form but, in this case, each diastereomer of the tether-bearing peptide would need to be evaluated in order to determine its efficiency in the ligation and acyl transfer steps. Of course, the complications resulting from the chirality of **80.2** could also easily be avoided by simply having two phenyl groups attached to the center α to the nitrogen atom, although such systems have not yet been examined by us.

2.2.3 Studies Involving Benzylic Tether 80.1

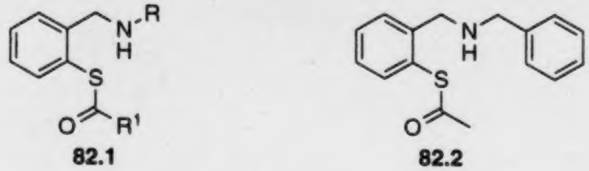
The following work represents exploratory studies on a difficult topic and includes a number of unsuccessful experiments and approaches used to try to synthesize particular compounds. We realized early on in our research that we would have to survey a large area and, therefore, in order to make rapid progress, we did not fully characterize all the compounds encountered, as was done for the research described in other parts of this thesis. The majority of the experiments that we conducted have been included here so that a record is available of all the avenues that have been explored, as this information is bound to be of value in the continuation of the work.

(a) Synthesis and Examination of a Model System for Acyl Transfer

Our initial investigations into peptide segment coupling by prior ligation and proximity-induced intramolecular acyl transfer focused on the use of the benzylic tether **80.1** (Scheme 80). As was mentioned previously, Kemp had examined related systems, in which an oxygen atom replaced the sulfur atom, and had noted some difficulties in the acyl transfer process. Hence, our first objective was to build a suitable model that would allow us to test whether or not acyl transfer would occur across this tether, at least in a simplified system. For this purpose we wanted a compound of the general type **82.1** (Scheme 82), which possesses all the features of our intended acyl transfer intermediates. The actual synthetic target that we chose is **82.2**.

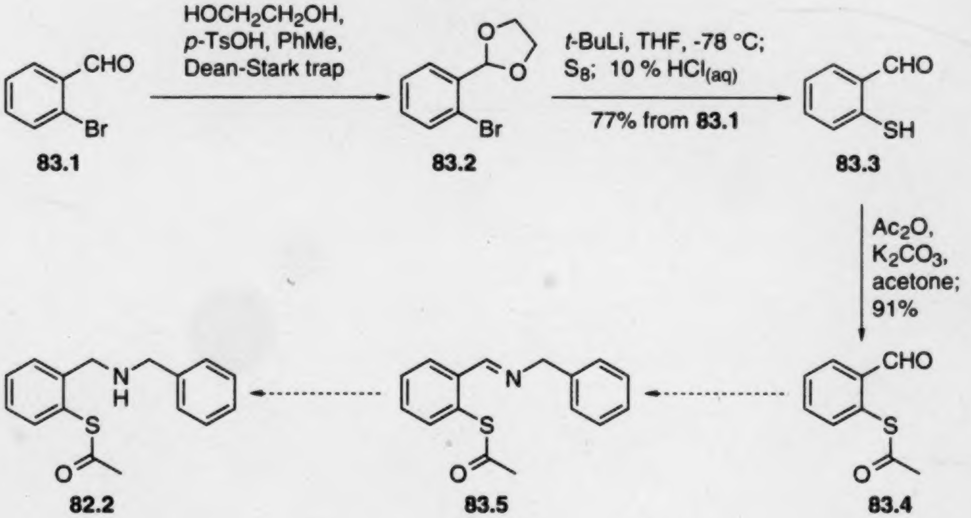
Our initial approach to model system **82.2** is outlined in Scheme 83. 2-Bromobenzaldehyde (**83.1**) was converted into cyclic acetal **83.2**, under standard conditions. This was then subjected to metal/halogen exchange, using *t*-BuLi,[96] to give the

Scheme 82



required lithiated species, which was treated with elemental sulfur to afford mercaptoaldehyde **83.3**, following hydrolysis of the acetal protecting group. Acylation of **83.3** proceeded in high yield to give **83.4**.

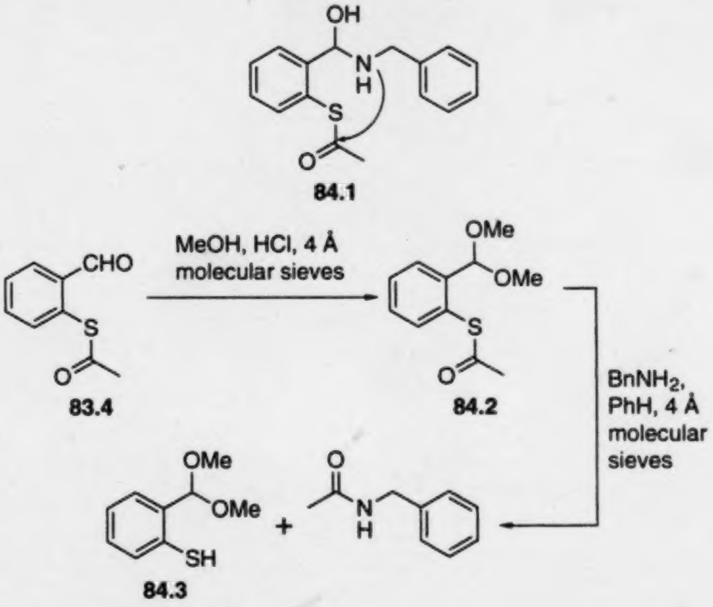
Scheme 83



With the required aldehyde in hand, we first tried to generate the desired model system **82.2** by reduction of an imine (**83.5**), generated *in situ*. This was tried by treating **83.4** with benzylamine and $NaCNBH_3$ in the presence of acidic methanol. However, only the corresponding dimethyl acetal (**84.2**, Scheme 84) was obtained from this experiment. We next explored a route whereby we would obtain the imine and, in a separate step, reduce it to the required amine. Unfortunately, all conditions tried for forming the desired imine from **83.4** also resulted in a substantial amount of benzylamide being formed.

Although at this point we wanted to try a different route to **82.2**, we wondered about the possibility that the benzylamide we had detected during imine generation, was formed by intramolecular acyl transfer from a hemiaminal (**84.1**, Scheme 84), generated *in situ* by reaction between benzylamine and the aldehyde of **83.4**. A similar reaction had been encountered by Kemp during one of his *Prior Amine Capture Approaches* (see Section 1.3.1). To test this possibility, dimethyl acetal **84.2** was generated by treatment of **83.4**, under the conditions shown, and was then reacted with benzylamine under the same conditions used previously to form the imine. Benzylamide was rapidly formed, and this fact implied that acyl transfer was probably proceeding intermolecularly, since **84.1** was not likely to have formed from the acetal **84.2**.

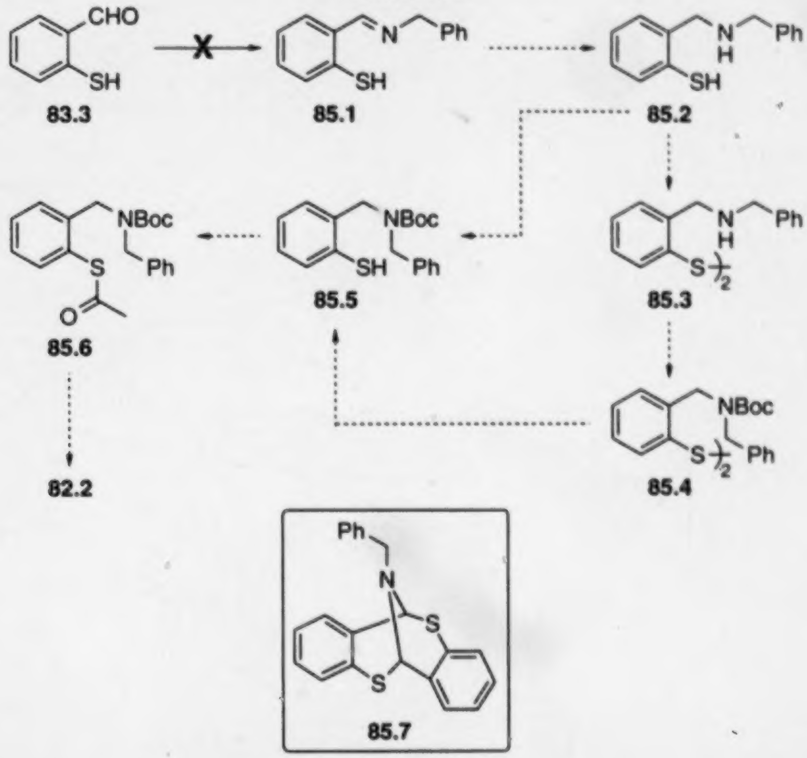
Scheme 84


OH
N
H
S
O
84.1
CHO
S
O
83.4
MeOH, HCl, 4 Å molecular sieves
OMe
OMe
S
O
84.2
BnNH2, PhH, 4 Å molecular sieves
OMe
OMe
SH
84.3
+
O
N
H

Our next approach to **82.2** is shown in Scheme 85. If we could generate thioamine **85.2**, then we intended to convert it into the corresponding *N*-Boc protected species **85.5**. Of course, the thiol group of **85.2** might have to be protected in order to access compound

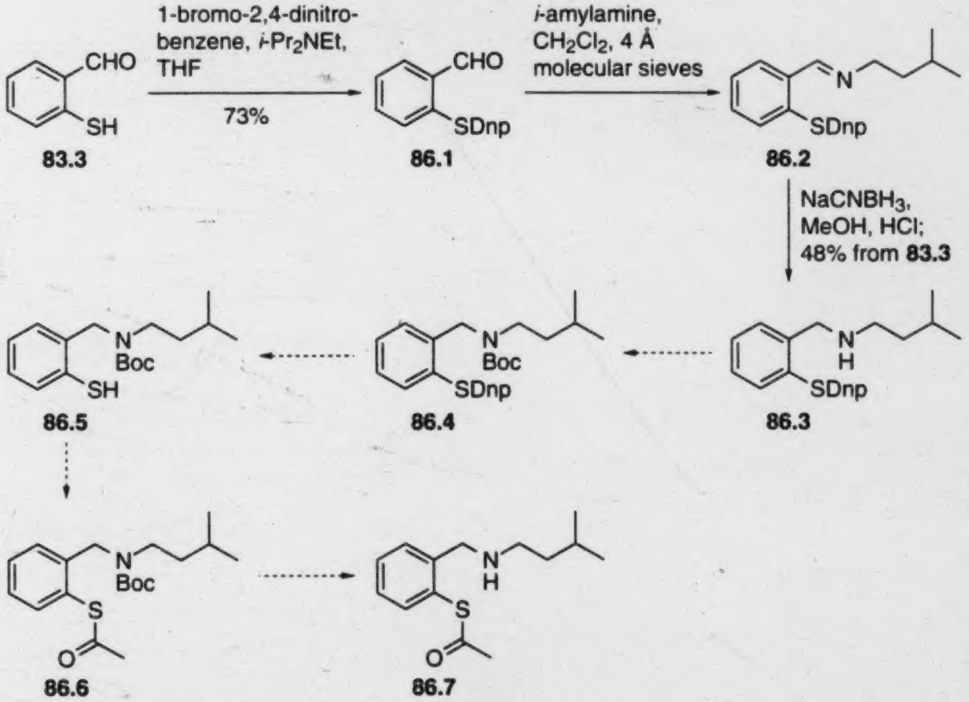
85.5. The desired model system (**82.2**) could then be generated from **85.5** by *S*-acylation and deprotection of the Boc-protected amine. In the event, this route failed very early on because attempted imine formation using **83.3** and benzylamine under dehydrating conditions, gave rise only to **85.7**, the product of an internal Mannich reaction.

Scheme 85



In order to prevent such an internal Mannich reaction, we decided to use an *S*-protected version of thioaldehyde **83.3**, in the hope that we could then effect imine formation. Hence, **83.3** was treated (Scheme 86) with 1-bromo-2,4-dinitrobenzene in the presence of Hunig's base to afford thioether **86.1**. This species was then treated with *i*-amylamine under dehydrating conditions, and gave imine **86.2**, which was subsequently reduced to amine **86.3** in low yield. Although at this point we could have tried the sequence of protection and deprotection steps shown in Scheme 86, we abandoned this approach for the time being because of the low yield for the imine formation/reduction, and decided to explore what appeared to be a more promising route that we were investigating simultaneously (see Schemes 87 and 88). We did, however, return to the approach of Scheme 86 in a modified form later on.

Scheme 86

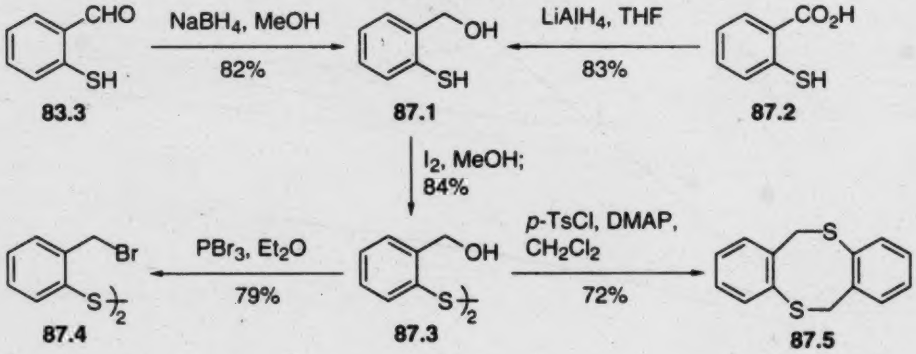

1-bromo-2,4-dinitrobenzene, i-Pr2NEt, THF
73%
i-amylamine, CH2Cl2, 4 Å molecular sieves
NaCNBH3, MeOH, HCl; 48% from 83.3
CHO
SH
SDnp
N
H
Boc
S
O
83.3
86.1
86.2
86.3
86.4
86.5
86.6
86.7

The first successful approach to model system **82.2** is that outlined in Schemes 87 and 88. This approach differed from those examined so far, in that nitrogen alkylation was achieved by displacement of a primary bromide, rather than by imine formation and reduction. The route began with reduction ($NaBH_4$) of thioaldehyde **83.3** to give benzyl alcohol **87.1**, which was then transformed into the corresponding symmetrical disulfide

(**87.3**) by oxidation. Benzyl alcohol **87.1** could also be obtained from commercially available 2-mercaptobenzoic acid (**87.2**) by $LiAlH_4$ reduction, and it was this route that we preferred because it avoids the preparation of **83.3**.

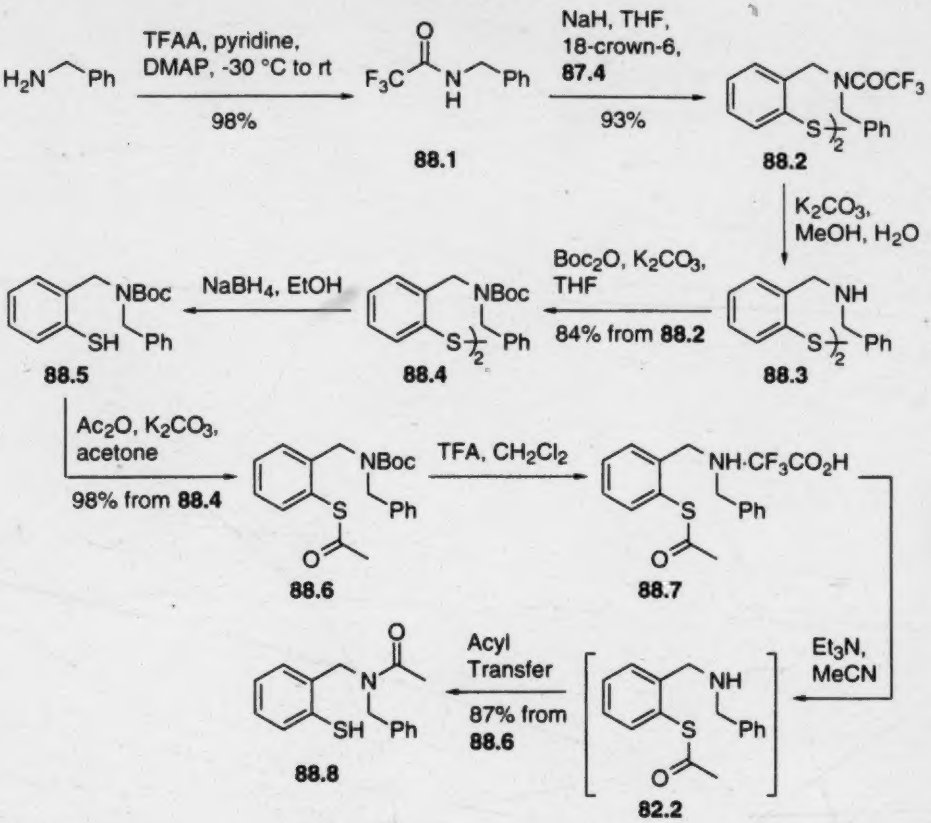
Conversion of diol **87.3** into the dibromide was attempted under various conditions, but only the use of PBr_3 gave a reasonable yield of the desired product (**87.4**). It should be mentioned that we also considered conversion of diol **87.3** into the ditosylate, as this species could potentially have served in place of the dibromide for nitrogen alkylation. However, attempted formation of the ditosylate under standard conditions resulted in the formation of compound **87.5**.

Scheme 87



Using dibromide **87.4**, trifluoroamide **88.1** (generated as shown, Scheme 88) was easily alkylated, and the product (**88.2**) of this reaction was then hydrolyzed to liberate amine **88.3**, which was subsequently protected as its *N*-Boc derivative **88.4**. Reduction of the disulfide was accomplished by treatment with $NaBH_4$, and took the route as far as thiol **88.5**. The thiol was then acylated to form **88.6**, which was deprotected using TFA, giving the TFA salt of **82.2**. Gratifyingly, after the considerable effort that went into making what appeared to be a very simple model system, we found that when **88.7** was treated with Et_3N, the acyl transfer product **88.8** was obtained within 10 min,

Scheme 88

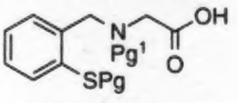


presumably via intermediate **82.2**.

(b) Synthesis of the Specially Derivatized Amino Acid

As a result of the work described above, we were able to establish that acyl transfer could, indeed, be carried out using benzyl tether **80.1**, at least in the simple system we had examined. Hence, our next short-term objective was to make a representative example of a specially derivatized amino acid (*cf.* **71.2**, Scheme 71), and show that it could be used in a conventional coupling reaction, and thus potentially be used to cap the *N*-terminus of a *C*-terminal peptide during solid phase peptide synthesis (*cf.* **71.1** + **71.2** → **71.3**). The general structure of the actual target we sought is **89.1** (Scheme 89).

Scheme 89

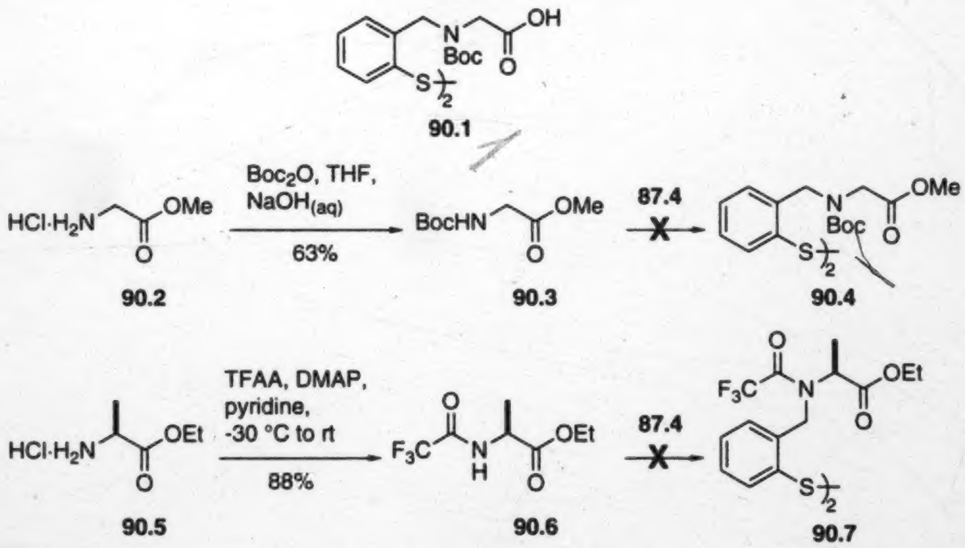


89.1

Pg, Pg^1 = protecting groups

The first approach we took for the preparation of the special amino acid was based on the successful synthesis of our model system for acyl transfer described above. Thus, the sulfur atom would be protected as a disulfide and the amine as a urethane (see **90.1**, Scheme 90). This route began with the preparation of a suitably protected glycine derivative (**90.2** → **90.3**) which we had hoped could be used directly in an alkylation reaction with dibromide **87.4** but, as it turned out, all efforts to effect such an alkylation were unsuccessful. We also tried to carry out the required transformation using a related trifluoroamide derivatized amino acid ester. In this case HClAlaOEt (**90.5**) was treated with TFAA to afford compound **90.6**, and this was then treated under various conditions with dibromide **87.4**. However, as was the case with BocGlyOMe (**90.3**), we were

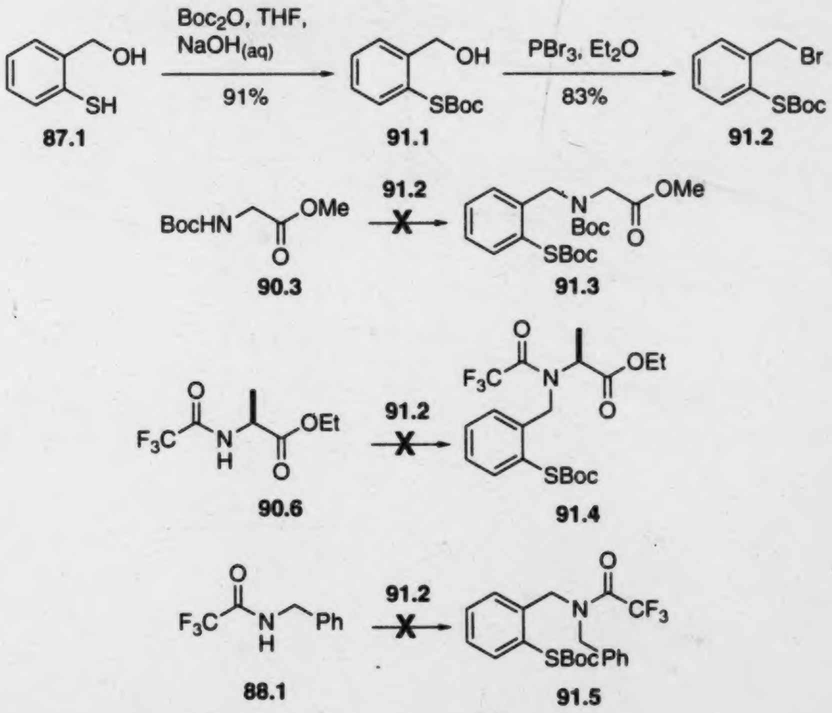
Scheme 90


90.1
Boc$_2$O, THF, NaOH$_{(aq)}$
63%
87.4
90.2
90.3
90.4
TFAA, DMAP, pyridine, -30 °C to rt
88%
87.4
90.5
90.6
90.7

unsuccessful. Apparently, alkylation using dibromide **87.4** is limited in scope.

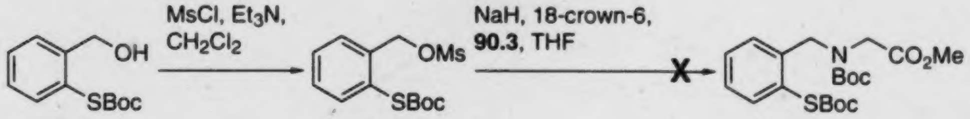
Since we were not successful at alkylating amino acid derivatives using dibromide **87.4**, we decided to vary the nature of the protecting group on sulfur. Hence, benzyl alcohol **87.1** was treated with Boc_2O in order to generate the *S*-Boc protected compound **91.1** (Scheme 91). Conversion of this species to the required bromide (**91.2**) was easily achieved by treatment with PBr_3. Several attempts (see Scheme 91) were made to use this bromide to alkylate three different amine derivatives, but in each case we obtained either a complex mixture, which did not show any clear evidence for formation of the desired product, or we recovered starting materials.

Scheme 91



We also prepared the corresponding mesylate (**92.1**) from benzyl alcohol **91.1**, and tried to use this in the alkylation of BocGlyOMe (**90.3**). However, when **90.3** was deprotonated and treated with **92.1**, the desired transformation did not occur, and only starting materials were recovered.

Scheme 92

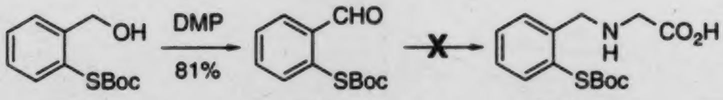


91.1 **92.1** **92.2**

At this point it was clear that we would have to make the tethered amino acid (*cf.* **89.1**) using a route that did not rely on nitrogen alkylation by an alkyl bromide. To this end, we returned to an earlier approach (see Scheme 86), albeit in a modified form, that we had abandoned in favor of the bromide-based alkylation route. In the present circumstances we planned to obtain a suitable sulfur-protected version of aldehyde **83.3** and, once again, attempt imine formation and reduction. We hoped that by varying the protecting group on sulfur, we would have greater success then when the 2,4-dinitrophenyl protecting group was used (*cf.* Scheme 86).

The first sulfur protecting group we examined was the Boc group. We made this choice for two reasons. First, it is easily removed by treatment with TFA and, secondly, it is the same group we intended to use on nitrogen, which meant that both protecting groups on the tethered amino acid could be liberated by a single, straightforward operation. The required *S*-Boc protected aldehyde was easily obtained by treatment of benzyl alcohol **91.1** with Dess-Martin reagent (Scheme 93). With aldehyde **93.1** in hand, we began to explore its conversion into an appropriate imine.

Scheme 93



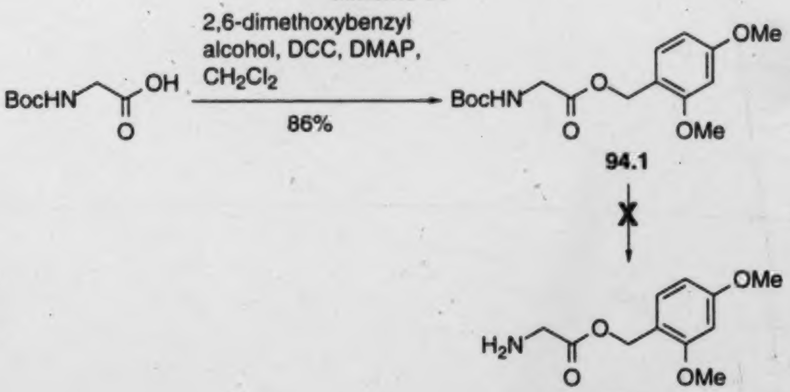
91.1 **93.1** **93.2**

The first attempt at imine formation was with glycine, but we were not able to achieve the required conversion (*cf.* **93.1** → **93.2**) under several conditions tried.

We next explored imine formation using ester-derivatized amino acids. Instead of simply using a methyl or ethyl ester, we wanted to examine the possibility of imine formation between aldehyde **93.1**, and an amino acid ester from which the corresponding free carboxylic acid could be obtained without relying on the normally harsh conditions of ester hydrolysis. We were concerned that, in a case other than glycine, hydrolysis of the ester might also cause epimerization at the α carbon of the amino acid component. We did not examine benzyl ester derivatives, because the conditions required for hydrogenolysis could potentially also hydrogenolyze the benzylamine tether. The esters that we considered were the 2,6-dimethoxybenzyl ester, which is cleaved under mild oxidative conditions using DDQ,[97] and the 2-naphthylmethyl ester, which is removed hydrogenolytically,[98] but under very mild conditions that do not affect benzyl esters and, presumably, benzylamines.[99]

The 2,6-dimethoxybenzyl ester protecting group was examined first (Scheme 94). We were unable to effect direct formation of the required ester using glycine and 2,6-dimethoxybenzyl alcohol, but we were able to couple BocGly with the alcohol under

Scheme 94



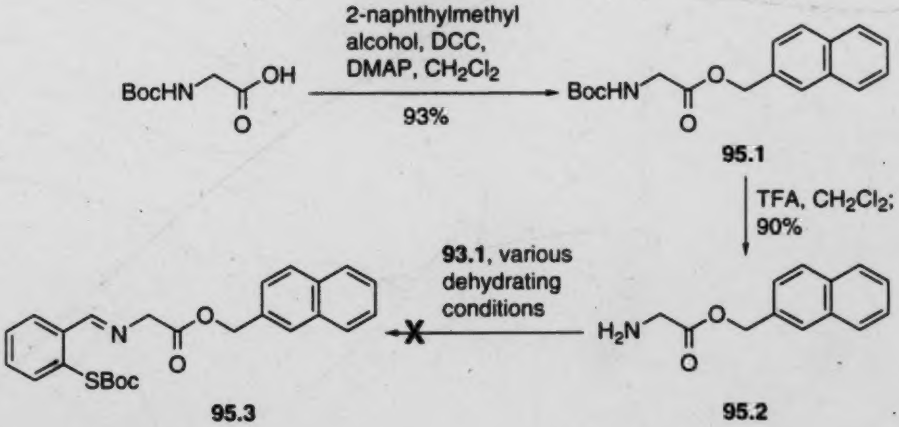
94.1

94.2

standard conditions, which gave *N*-Boc protected ester **94.1**. Unfortunately, our attempts to remove the nitrogen protecting group from this compound using 10% (v/v) TFA in CH_2Cl_2 gave only a complex mixture; apparently the 2,6-dimethoxybenzyl ester was too acid-labile for our purposes. We did not explore the corresponding route using FmocGly at the time, an approach which would have required base-mediated deprotection of the nitrogen, but instead chose to examine the 2-naphthylmethyl ester.

The 2-naphthylmethyl ester of glycine (**95.2**) was prepared as outlined in Scheme 95. BocGly and 2-naphthylmethyl alcohol were coupled under standard conditions to give the fully protected glycine species **95.1**. Treatment of this ester with TFA removed the nitrogen protecting group, giving the desired amino ester (**95.2**) without complications. Having a suitable glycine ester available, we attempted imine formation using *S*-Boc protected aldehyde **93.1** under various conditions, but all that we could obtain was, at best, a trace amount of what might have been the imine — as determined by the presence of small peak in the ^{1}H NMR spectrum of the crude material at approximately 8.2 ppm.

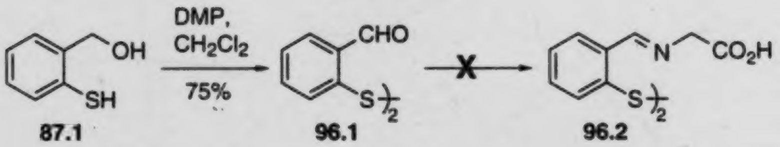
Scheme 95


2-naphthylmethyl alcohol, DCC, DMAP, CH2Cl2
93%
BocHN
OH
O
95.1
TFA, CH2Cl2; 90%
93.1, various dehydrating conditions
H2N
95.2
N
SBoc
95.3

It was not clear why the *S*-Boc protected aldehyde should be so unreactive towards imine formation. Initially we supposed that the origin of the low reactivity might be due to steric bulk caused by the sulfur protecting group. However, we had already established that a different and somewhat bulky *S*-protected version of aldehyde **83.3** (Scheme 83) did react to give an imine (**86.1** → **86.2**, Scheme 86), and so we are not convinced by the steric argument. Moreover, we subsequently established (see later) that when aldehyde **83.3** was protected on sulfur with a bulky *t*-Bu or Fm group, imine formation occurred readily.

We considered the possibility that the diminished reactivity of the aldehyde toward imine formation was rooted in electronic, rather than steric factors, although it was not clear why this should be so [in all the successful cases of imine formation (see **86.1** → **86.2**, Scheme 86, and later) the sulfur was in the form of a thioether]. To examine this matter further, we prepared the disulfide protected version of aldehyde **83.3**, and attempted to use it for imine formation (Scheme 96). Interestingly, as was the case with the *S*-Boc protected version of the aldehyde, the desired reaction did not occur. Apparently, having the thiol protected as a thioether is critical to successful imine formation.

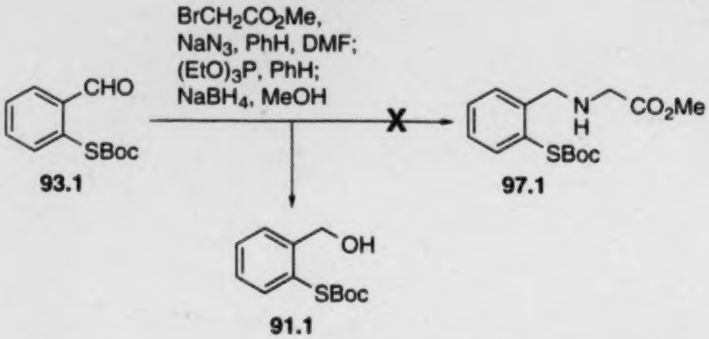
Scheme 96



We also explored a decidedly more aggressive approach to imine formation based on an aza-Wittig reaction, which is shown in Scheme 97. However, all that was recovered from this experiment was *S*-Boc protected benzyl alcohol **91.1**.

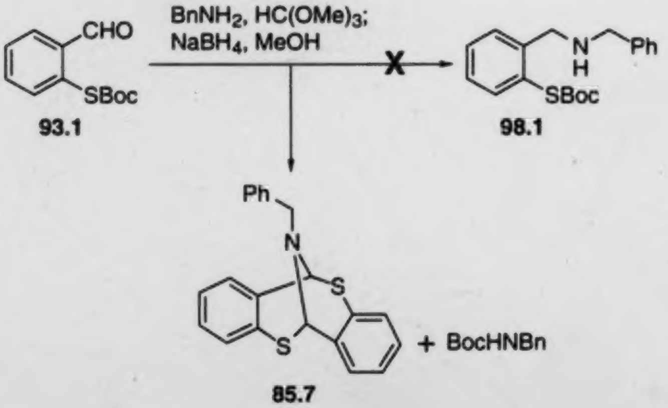
We then resorted to the use of GlyOMe as the amine component in a final attempt to make an amino acid-based imine from aldehyde **93.1**. When **93.1** was treated with GlyOMe using $(MeO)_3CH$ as a dehydrating agent, and the product of that reaction treated directly with $NaBH_4$, all that was isolated was the undesired aldehyde reduction product

Scheme 97



91.1. Interestingly, when treated under the same conditions, but using benzylamine instead of GlyOMe, aldehyde **93.1** reacted to give **85.7**, and Boc protected benzylamine (Scheme 98). Compound **85.7** had been observed earlier during attempted imine formation from 2-mercaptobenzaldehyde (**83.3**) and benzylamine. Apparently, in the present case, benzylamine and **93.1** were undergoing Boc exchange [either intermolecularly, or intramolecularly via an initially formed hemiaminal (*cf.* **84.1**, Scheme 84)] to give aldehyde **83.3**, which then underwent an internal Mannich reaction with additional benzylamine. No matter what the actual mechanism is that leads to formation of the observed products, it was clear at this point that use of *S*-Boc protected aldehyde **93.1** was not likely to be effective for alkylation of amino acid derivatives. Even if we could

Scheme 98



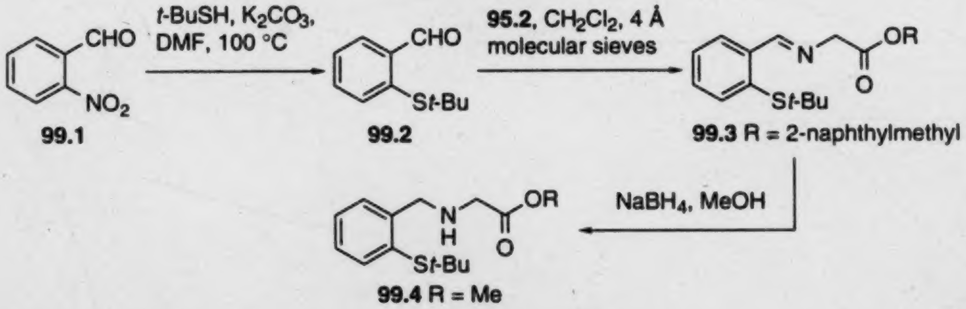
establish conditions in which the amino acid species and **93.1** would react, the result was expected to be similar in outcome to that just described, namely Boc transfer from sulfur to nitrogen and subsequent Mannich reaction.

As we still wanted to be able to make the required tether-bearing amino acid derivatives by an imine formation/reduction route, we turned our attention to a different sulfur protecting group, and we chose to examine the *t*-butyl group. We had already observed imine formation using a thioether-protected version of aldehyde **83.3**, and hoped that we would be successful in the present case as well. Hence, the actual target that we sought was (**89.1**, Pg = *t*-Bu, Pg^1 = Boc, Scheme 89).

Removal of the *t*-butyl group could potentially be achieved by dissolving metal (Na/$NH_{3(l)}$), or by treatment with TFA and $Hg(OAc)_2$, followed by reduction of the resulting mercaptide. The later conditions were preferable, in that they would also cleave the Boc group that we planned to use to protect the nitrogen atom. Unfortunately, these conditions were considerably more cumbersome than simple treatment with TFA, which would have sufficed had we been able to generate the *S*-*N*-bisBoc protected system (*cf.* **89.1**, Pg, Pg^1 = Boc, Scheme 89).

The *S*-*t*-Bu protected aldehyde was obtained from commercially available 2-nitrobenzaldehyde by treatment with *t*-BuSH under basic conditions (**99.1** → **99.2**, Scheme 99). When this species was treated with amino ester **95.2** in the presence of

Scheme 99

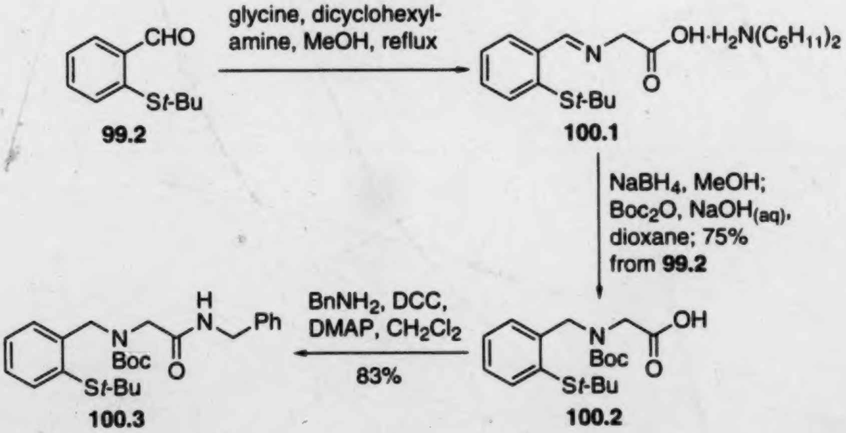

CHO
NO2
99.1
t-BuSH, K2CO3, DMF, 100 °C
CHO
St-Bu
99.2
95.2, CH2Cl2, 4 Å molecular sieves
N
OR
O
St-Bu
99.3 R = 2-naphthylmethyl
NaBH4, MeOH
N
H
OR
O
St-Bu
99.4 R = Me

molecular sieves, an approximately 6:4 mixture of imine **99.3** to aldehyde **99.2** was obtained, as judged by the ^{1}H NMR of the crude material. The imine was treated with $NaBH_4$ in MeOH in order to generate the amine, but we obtained instead the amine corresponding to ester exchange with the solvent (**99.4**). In an effort to inhibit ester exchange, the reduction was repeated, but this time using the more bulky solvent *i*-PrOH in place of MeOH. However, the product was still that of ester exchange (**99.4** R = *i*-Pr).

As it turned out, we were able to generate the carboxylic acid that we desired from aldehyde **99.2** directly, without recourse to an esterified version of an amino acid. This was achieved by treating the aldehyde with glycine, under the conditions shown in Scheme 100, to give imine salt **100.1**, which was subsequently reduced to the corresponding amine, and then protected on nitrogen (**100.1** → **100.2**).

With the required carboxylic acid (**100.2**) in hand, we were then able to establish that it could, indeed, be used in a conventional coupling reaction. This was done by treating the acid with benzylamine under standard coupling conditions (**100.2** → **100.3**). The fact that this reaction proceeds smoothly and in high yield, suggested that our specially derivatized amino acids would potentially be applicable in the final step of solid phase synthesis of the peptide segments that we ultimately required.

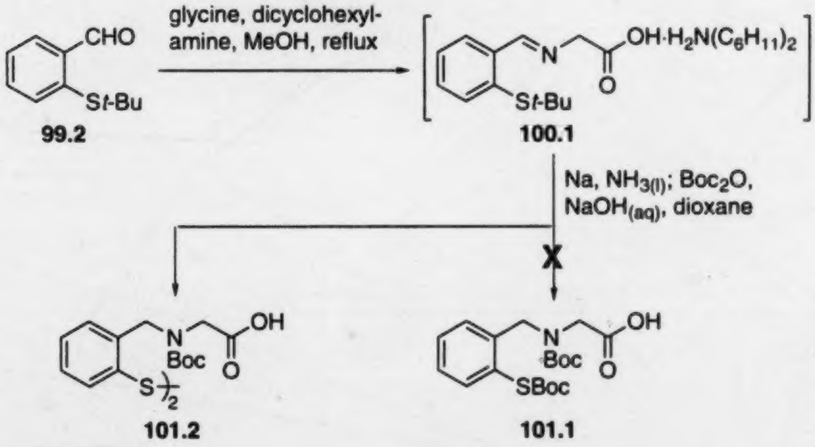
Scheme 100



As mentioned in Section 2.2.2a, from the outset of our research we wanted to be able to obtain our final objectives using the simplest chemistry possible. Relative to *S*-Boc protection, sulfur protection using a *t*-Bu group is considerably more awkward with respect to the ease and cleanliness of its removal. Therefore, although we had achieved our second short term objective, we decided to make a brief detour to see if we could use **100.2**, or a related species, to generate an *S,N*-bisBoc protected tether-bearing amino acid.

Our first attempt at doing this involved treating imine **100.1**, with $Na/NH_{3(l)}$, followed by Boc_2O. We hoped that the fully deprotected thioamine would be obtained from **100.1** under the reductive conditions, and that both the amine and thiol substituents would then react with Boc_2O to give **101.1**. As it turned out, exposure of the imine to $Na/NH_{3(l)}$ did serve both to reduce the imine and liberate the free thiol, but only the amino group reacted with Boc_2O, whereas the thiol formed a disulfide (**101.2**).

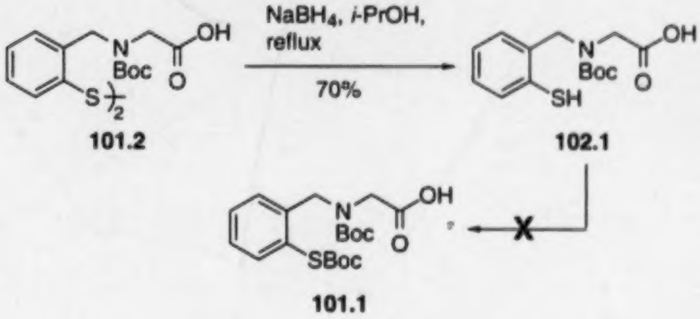
Scheme 101


CHO
S*t*-Bu
99.2
glycine, dicyclohexylamine, MeOH, reflux
N
OH·$H_2N(C_6H_{11})_2$
O
S*t*-Bu
100.1
Na, $NH_{3(l)}$; Boc_2O, $NaOH_{(aq)}$, dioxane
X
N
Boc
OH
O
S
2
101.2
N
Boc
OH
O
SBoc
101.1

We next explored the possibility of generating the *S*-Boc species from the corresponding thiol, in a system that already had the amine in the required protected form. This process began with reductive cleavage of the disulfide bond of **101.2** with $NaBH_4$ in refluxing *i*-PrOH. Reduction of the disulfide could not be affected using either refluxing

MeOH or EtOH, the higher temperature of boiling *i*-PrOH being essential. Several attempts were then made to protect the thiol and obtain the desired *S,N*-bisBoc protected species, but our efforts (Scheme 102) were uniformly unsuccessful, resulting in either the recovery of starting material or the corresponding symmetrical disulfide.

Scheme 102



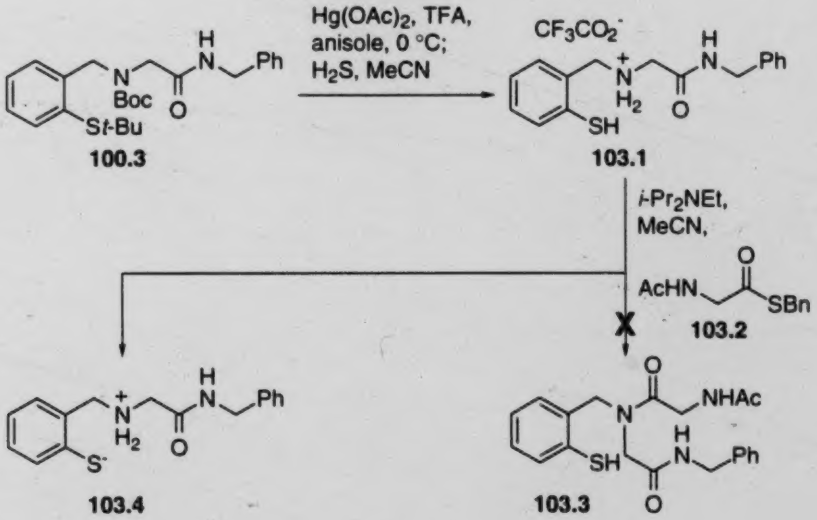
For the time being, we chose not to pursue the *S,N*-bisBoc protected species further, but instead examined how the *N*-Boc, *S*-*t*-Bu-protected compound we had made would respond in the deprotection, ligation, and acyl transfer sequence that we wanted to apply.

(c) Deprotection, Ligation and Intramolecular Acyl Transfer

Up to this point, we had not formally addressed the actual ligation aspect of our approach to segment coupling. In order to do this we would first have to obtain a suitably deprotected version of the systems that we had been trying to synthesize.

We first evaluated the deprotection, ligation, and acyl transfer sequence using compound **100.3**, since we already had a small amount available. Deprotection of **100.3** (Scheme 103) was carried out by treatment with cold TFA and anisole in the presence of $Hg(OAc)_2$, followed by exposure to H_2S. This sequence gave a compound whose ^1H NMR characteristics were consistent with the expected TFA salt **103.1**; however, we were unable to purify the material. As a result, we repeated the deprotection sequence (**100.3** → **103.1**), and then attempted the ligation and acyl transfer reactions using the crude material, by dissolving it in MeCN and then adding *i*-Pr_2NEt, which caused immediate formation of a white precipitate. Thioester **103.2** was then added but, even after a stirring period of 11 h, it was clear that the reaction was not progressing, as judged by the fact that the thioester was not being consumed (the reaction was monitored by TLC). Subsequently, it was established that what was actually occurring, was that the TFA salt was being neutralized by addition of base, and that this caused the zwitterionic species **103.4** to form, which is insoluble in the solvent being used. The zwitterion precipitated from solution without further reaction. Although we were unsuccessful in carrying out the ligation and acyl transfer reactions using the deprotected material, the experiments at least furnished us with a straightforward preparation of pure **103.4**, which we could use in further investigations.

Scheme 103



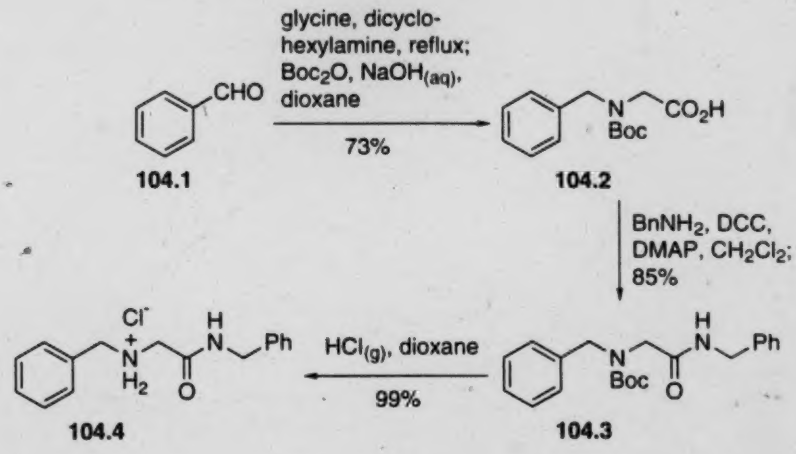
As it turned out, compound **103.4** was insoluble in water, and in all of the standard organic solvents we tried, except DMSO. Hence, the ligation and acyl transfer process was attempted using this solvent. To this end, equimolar amounts of **103.4** and thioester **103.2** were dissolved in DMSO-d_6, and *i*-Pr$_2$NEt was added to the mixture. The reaction was monitored by ^{1}H NMR, which showed the disappearance of the *N*-acetyl methyl signal corresponding to the starting material **103.2**, and the appearance of additional signals in that region. Within 15 h, no further change in the NMR spectrum was observed, but it was clear that the reaction had not gone to completion, since signals corresponding to the thioester were still present. The product was isolated chromatographically and, on the basis of ^{1}H and ^{13}C NMR, determined to be the desired product **103.3**.

At this point we were interested in trying to confirm if the coupling reaction we had observed was indeed the result of an actual ligation and acyl transfer process, or if it was simply the result of intermolecular acyl transfer from thioester **103.2** to the secondary amino group of **103.1**, once the amine had been neutralized. Hence we conducted two control experiments as follows.

The first of these was intended to establish if the ligation reaction had actually occurred. This required preparation of a suitable analog of **103.1** lacking the thiol substituent, and the obvious choice was compound **104.4**, which was made as outlined in Scheme 104. Benzaldehyde was treated with glycine under the conditions that we had established earlier, and this gave *N*-Boc protected amino acid **104.2**. This was then coupled with benzylamine (**104.2** → **104.3**), and the product was treated with HCl to give salt **104.4**. The amine was generated *in situ* by neutralization with *i*-Pr$_2$NEt, and this was done in the presence of the thioester **103.2**. The reaction mixture was monitored by ^{1}H NMR, but no reaction had occurred, even after several days. This result strongly suggests that the ligation reaction had indeed occurred in the previous example we had studied (**103.1** → **103.2**, Scheme 103).

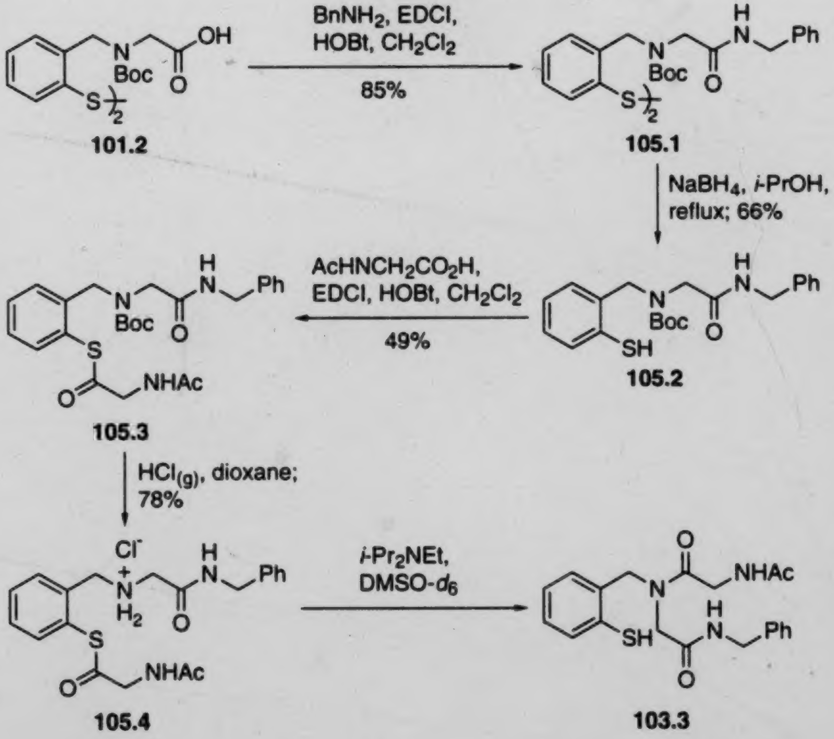
On this basis, it seemed reasonable to assume that acyl transfer had then proceeded in an intramolecular sense. However, in order to support this assumption, the presumed

Scheme 104


glycine, dicyclohexylamine, reflux;
Boc$_2$O, NaOH$_{(aq)}$, dioxane
73%
CHO
104.1
N
Boc
CO$_2$H
104.2
BnNH$_2$, DCC, DMAP, CH$_2$Cl$_2$;
85%
H
N
Ph
O
104.3
HCl$_{(g)}$, dioxane
99%
Cl$^-$
+
N
H$_2$
104.4

ligation system was generated by an independent route, and then tested for its ability to undergo acyl transfer. The synthesis of the system required is shown in Scheme 105.

Scheme 105

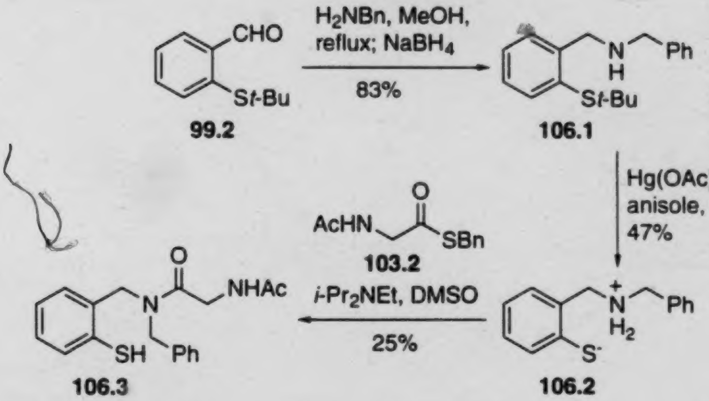

OH
N
Boc
O
S
2
101.2
BnNH$_2$, EDCI, HOBt, CH$_2$Cl$_2$
85%
H
N
Ph
105.1
NaBH$_4$, *i*-PrOH, reflux; 66%
SH
105.2
AcHNCH$_2$CO$_2$H, EDCI, HOBt, CH$_2$Cl$_2$
49%
NHAc
105.3
HCl$_{(g)}$, dioxane;
78%
Cl$^-$
+
H$_2$
105.4
i-Pr$_2$NEt, DMSO-d_6
103.3

Disulfide **101.2** was coupled with benzylamine to give **105.1**, which was then reduced to the corresponding thiol by treatment with $NaBH_4$ (**105.1** → **105.2**). The thiol was acylated under standard coupling conditions (**105.2** → **105.3**) and the product of this reaction was treated with HCl to remove the Boc group (**105.3** → **105.4**), giving HCl salt **105.4**. An ^1H NMR experiment was carried out in which **105.4** was dissolved in DMSO-d_6 and exposed to *i*-Pr_2NEt. Examination of the ^1H NMR spectra of the reaction mixture indicated that, under these conditions, the desired acyl transfer product (**103.3**) appeared to have formed within 3 min, supporting the assumption that acyl transfer was occurring intramolecularly.

At this point we started to make preparations for examining the coupling process using a variety of tether-bearing amino acids and thioesters. In our previous work, the ligation and acyl transfer experiments had been conducted on a small scale, since we had had only a very small amount of material available. We now planned to prepare larger quantities of a simpler version of the tether-bearing amino acid, and then try to optimize the conditions for the coupling process before applying it to more complex systems.

The compound we had in mind for this purpose is **106.1**, which was prepared as shown in Scheme 106. Aldehyde **99.2** and benzylamine were combined in refluxing MeOH to generate the corresponding imine, which was then reduced to amine **106.1**. Deprotection of the thiol was carried out as before to give zwitterion **106.2**. This species was combined with thioester **103.2** in the presence of *i*-Pr_2NEt, and gave a low yield of a compound whose structure was consistent with that of the desired product (**106.3**), as determined by ^1H and ^{13}C NMR. The NMR spectra were rather complex, due to the fact that the product exists as a rotamer mixture at room temperature, and so we were not able to establish from this data whether our product was in the form of the thiol, or in the form of a symmetrical disulfide, and we could not obtain a satisfactory mass spectrum. If disulfide formation was occurring, then that could explain why we had obtained such a low yield. We sought, therefore, to generate the thiol and symmetrical disulfide by an independent

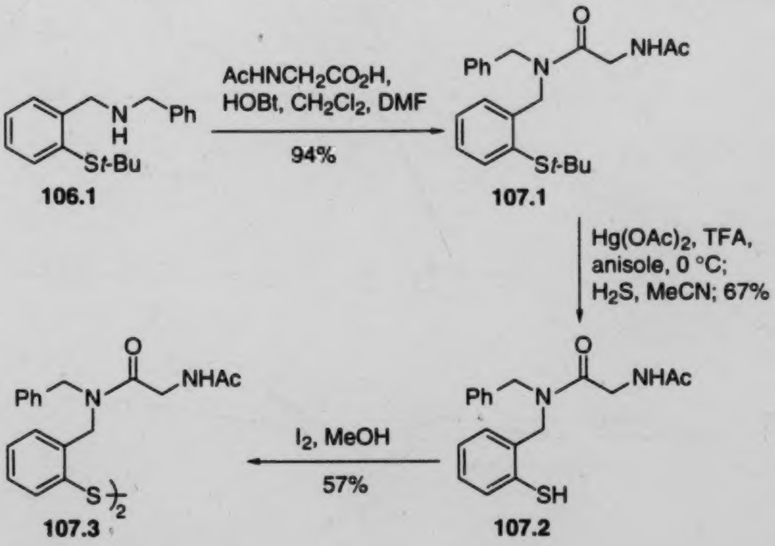
Scheme 106



route so that we would have available reference NMR spectra.

Synthesis of these compounds began with amine **106.1**, which underwent EDCI-mediated acylation with *N*-acetylglycine (**106.1** → **107.1**, Scheme 107), and then thiol deprotection (**107.1** → **107.2**) gave **107.2**. This was treated with I_2 to afford the required disulfide (**107.3**). Oddly, neither the ^{1}H NMR spectrum of the thiol (**107.2**) nor the *symmetrical* disulfide (**107.3**) matched that of the product we had obtained from the

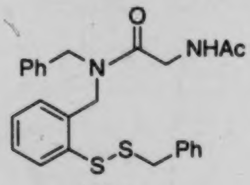
Scheme 107



coupling reaction (**106.3**, Scheme 106), although the spectra were all similar.

Puzzled by this result, we repeated the coupling reaction between **106.2** and **103.2**, but this time in the presence of PhSH, which Kent has suggested inhibits disulfide formation in related systems (see Section 1.5.1a). The major product obtained from this experiment appeared to be mixed disulfide **108.1**, whose structure was established on the basis of both NMR and high resolution mass spectra. It was surprising that this compound should have formed as the major product. First of all it has been stated in the literature that the thiol additive suppresses disulfide formation of this type. Secondly, since PhSH was present in the reaction mixture in a ten-fold excess relative to the thioester, if an unsymmetrical disulfide were to form, it would be expected to form from PhSH, not BnSH.

Scheme 108



108.1

With the situation regarding the ligation and acyl transfer aspects of our approach to segment coupling based on benzylic tether **80.1** (Scheme 80) still unclear, we were forced to stop work on it at this point, since, much to our disappointment, what was essentially the same approach that we were investigating, appeared in the literature[100] from a group at the Scripps Research Institute, led by Dawson, a former student of Kent's. In this publication, Dawson *et al.* demonstrated that benzylic tether **80.1** could indeed be used to couple small peptides. However, his approach for introducing the tether into the *N*-terminal segment was different from our intended approach, and had not worked very well. The authors also did not attempt to cleave the tether following the coupling reaction, and so obtained coupled products with a modified peptide backbone.

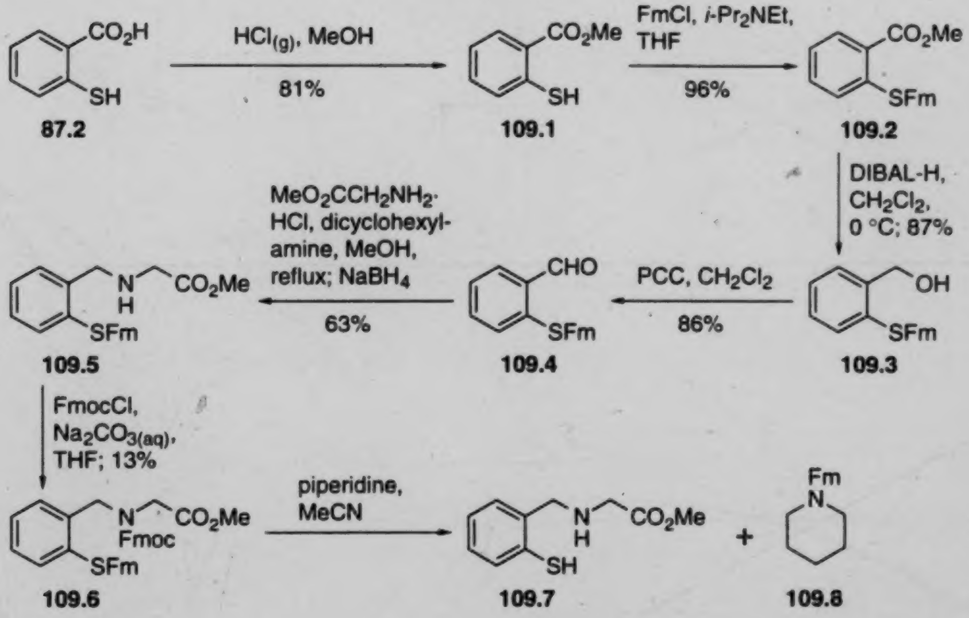
As a result of the Dawson publication, we decided to abandon the approach we had been investigating, in favor of examining the use of benzylic tether **80.2** as a segment coupling device. While our work on ligation and acyl transfer using tether **80.1** was being conducted, we were also carrying out preliminary work for another of our objectives, namely extending the approach so that it could be used in an iterative fashion. Since the principles of this work are potentially applicable to the new tether as well, the work related to iteration will be described briefly before embarking on a discussion of our modified approach to ligation and acyl transfer.

(d) Studies Towards an Iterative Approach

Our plans for modifying the approach to peptide segment coupling that we had been working on, so that it could be applied in an iterative sense, were described earlier in section 2.2.1b. The first short-term objective we had towards this goal, was to establish a synthetic route to compounds of the type represented by **72.2** (Scheme 72). Following this, we simply wanted to determine if a deprotection sequence could be conducted without adversely effecting the tethered system.

The actual synthetic target we chose was **109.6**, and the route we developed to obtain it is outlined in Scheme 109. 2-Mercaptobenzoic acid (**87.2**) was esterified to give **109.1**, and the thiol group of this compound was then protected by treatment with FmCl, in the presence of base (**109.1 → 109.2**). DIBAL-H reduction afforded benzyl alcohol **109.3**, which was oxidized to the corresponding aldehyde (**109.4**) using PCC. At this point the standard imine formation/reduction protocol that had served us previously was carried out, giving amino ester **109.5** in acceptable yield (63%). Protection of the amine moiety in **109.5** could be achieved using FmocCl and Na_2CO_3 (**109.5 → 109.6**), but the yield was very low. We made no effort to optimize this reaction. Having the model system in hand, we attempted its deprotection to the parent thioamine. This was achieved in a very clean and efficient manner by treatment of **109.6** with piperidine. The ^{1}H NMR spectrum of the crude material was very clean, showing only the desired product (**109.7**) and the expected amine byproduct **109.8**, which we were not able to separate.

Scheme 109



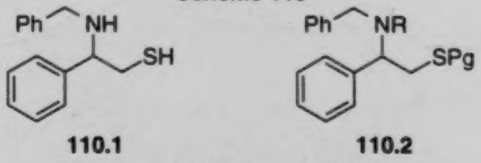
The sequence of reactions just described clearly showed that the desired Fm/Fmoc protected tether-bearing amino acids could be obtained, although the nitrogen protection step would have to be improved. Our experiments also showed that such tether-bearing amino acids would potentially function in the required manner in the iterative process, when the *N*-terminal amino acid required deprotection.

2.2.4 Studies Involving Benzylic Tether 80.2

(a) Synthesis of a Model System for Ligation and Acyl Transfer

Our investigation into the use of benzylic tether **80.2** as a segment coupling device, began with the synthesis of a model system that we could use in an actual ligation and acyl transfer procedure. The target we chose for this purpose is **110.1**, which we planned to make from a suitably protected analog (**110.2**).

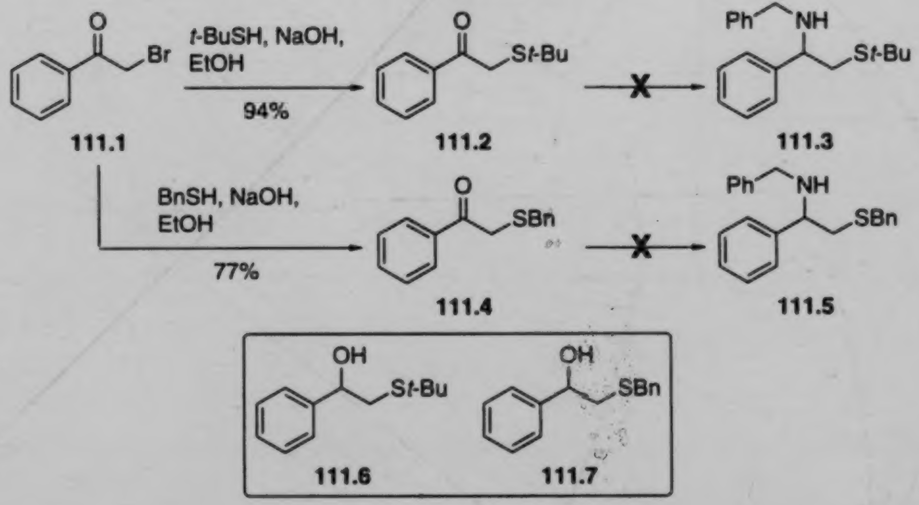
Scheme 110



R = H or acid-labile protecting group
Pg = acid labile protecting group

The first protected analog of **110.1** that we sought is the *S*-*t*-Bu species **111.3**, and the initial approach we took is outlined in Scheme 111. *t*-BuSH was *S*-alkylated by treatment with 2-bromoacetophenone[101] (**111.1**) in the presence of base to give compound **111.2**. Conversion of this species into amine **111.3** was attempted in several ways, but in all cases only the product of ketone reduction (**111.6**) was obtained. We assumed that the reason we were unable to form an imine from **111.2** was because the ketone was too sterically encumbered. For this reason, we prepared the potentially less sterically restricted

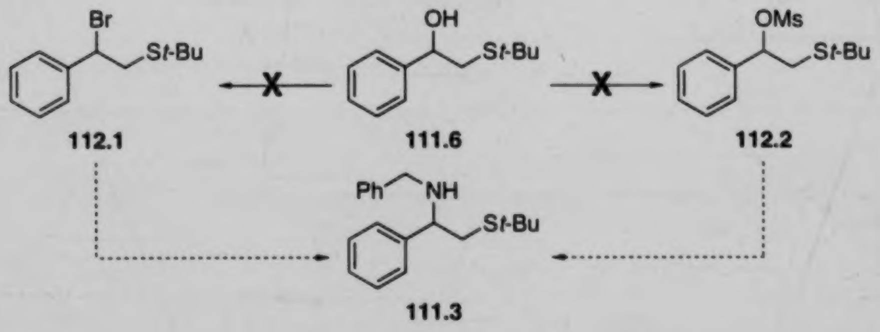
Scheme 111



species **111.4** by alkylation of benzylmercaptan.[101] Like the *S*-*t*-Bu protecting group, the *S*-benzyl group can be cleaved by treatment with TFA and $Hg(OAc)_2$, followed by reduction of the resulting mercaptide. Unfortunately, attempted reductive amination of **111.4** using benzylamine was not successful under a variety of conditions, and only **111.7** was isolated.

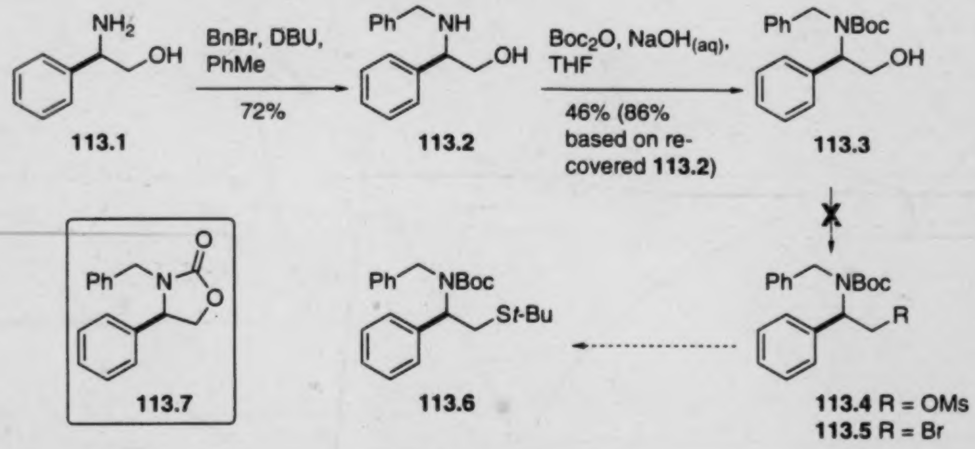
We next took a slightly modified approach to compound **111.3**, which involves *N*-alkylation using an appropriate bromide or mesylate, as shown in Scheme 112. However, when we attempted to form either the mesylate (**112.2**) or the bromide (**112.1**) from benzyl alcohol **111.6**, we recovered only starting material.

Scheme 112



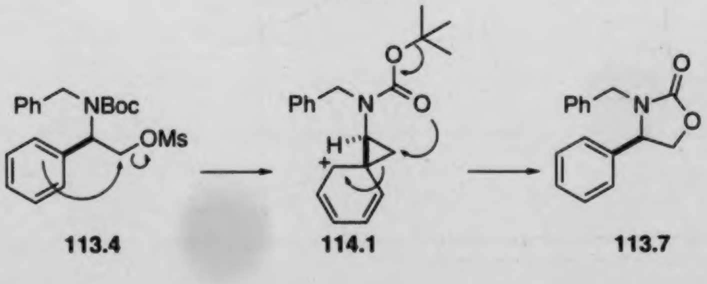
We next explored a route to a protected analog of **110.1** which began with (*R*)-phenylglycinol (**113.1**, Scheme 113). (*R*)-Phenylglycinol was treated with BnBr in the presence of DBU to obtain the *N*-alkylated material **113.2**,[102] which was then protected on nitrogen to give compound **113.3**. Mesylate formation from this species was then tried, but we obtained only oxazolidinone **113.7**. Formation of this species is assumed to result from displacement of the initially formed mesylate by the phenyl substituent to give **114.1** (Scheme 114), which is then trapped intramolecularly by the urethane carbonyl, eventually giving the observed product. Attempted conversion of **113.3** into bromide

Scheme 113



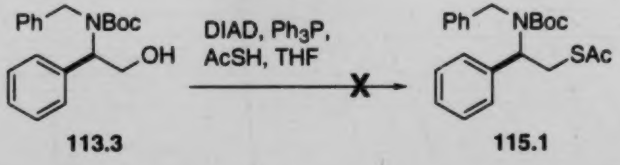
113.5 (Scheme 113), also resulted only in the formation of **113.7**, presumably by a similar mechanism.

Scheme 114



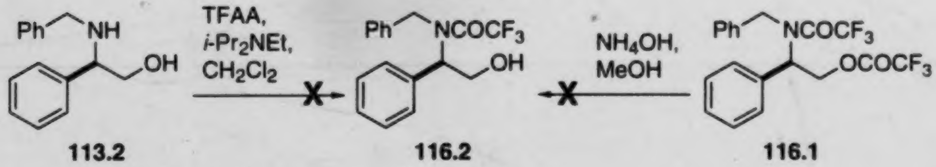
We then tried to carry out a Mitsunobu reaction on alcohol **113.3**, using thioacetic acid[103] (Scheme 115), but this experiment gave only a complex mixture, with no clear indication that the desired product had formed.

Scheme 115



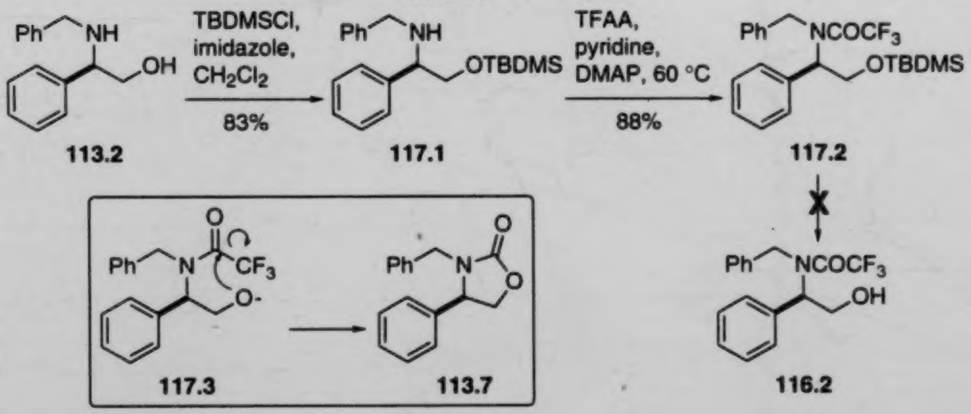
In an effort to prevent the rearrangement shown in Scheme 114, we tried to vary the nature of the nitrogen protecting group. Hence, alcohol **113.2** was treated with one molar equivalent of TFAA in the presence of *i*-Pr_2NEt in order to generate amide **116.2** (Scheme 116). However, all that was obtained under these conditions was the diacylated product **116.1**, and unreacted starting material. We decided, therefore, to attempt selective hydrolysis[104] of **116.1** to the desired product (**116.2**), but recovered only **113.2** instead.

Scheme 116



In order to prevent unwanted diacylation of **113.2**, its hydroxyl group was protected as a silylether prior to conducting the acylation reaction. The silylether was formed under standard conditions (**113.2** → **117.1**, Scheme 117), and was subsequently converted into amide **117.2**. Unfortunately, when this species was subjected to typical conditions used for removal of the hydroxyl protecting group, only **113.7** was obtained.

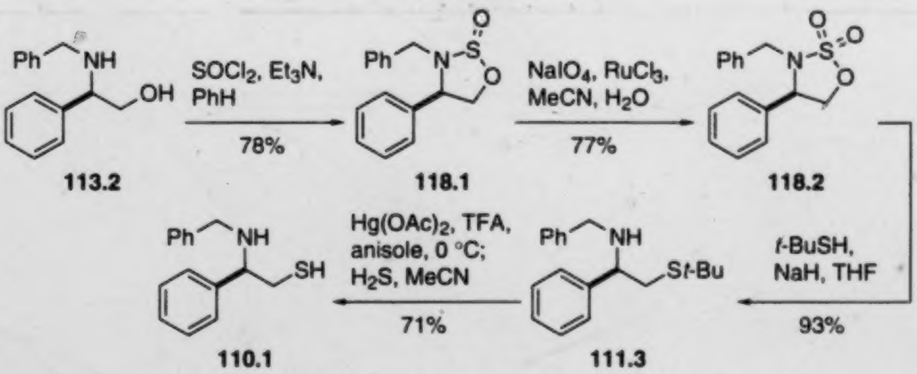
Scheme 117



In this case, the alkoxide (**117.3**) generated during the deprotection reaction must have attacked the carbonyl of the amide, expelling trifluoromethane anion and giving the observed product. We did not try TBAF in THF-AcOH.

The successful approach to **110.1** is shown in Scheme 118. Compound **113.2** was treated with $SOCl_2$ and Et_3N[105] which gave **118.1** as a mixture of diastereomers, and these were then oxidized[106] to **118.2**. Compound **118.2** was used to alkylate *t*-BuSH, giving thioether **111.3** in excellent yield. Treatment of the thioether under standard deprotection conditions gave the required model compound **110.1** in good yield.

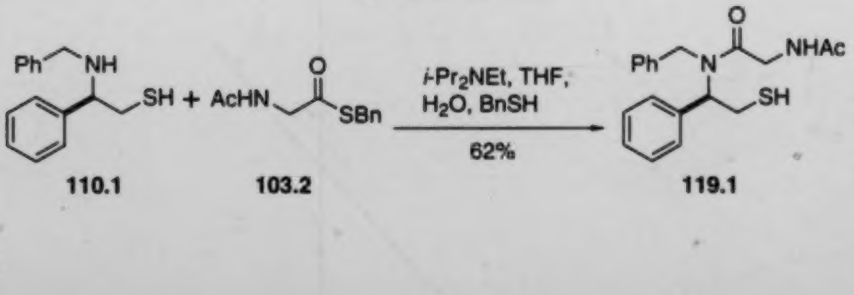
Scheme 118



(b) Ligation and Acyl Transfer

With model compound **110.1** in hand, we began to investigate how it would function in ligation and acyl transfer reactions. To this end, **110.1** was combined with thioester **103.2** under the conditions shown in Scheme 119, which produced the desired

Scheme 119



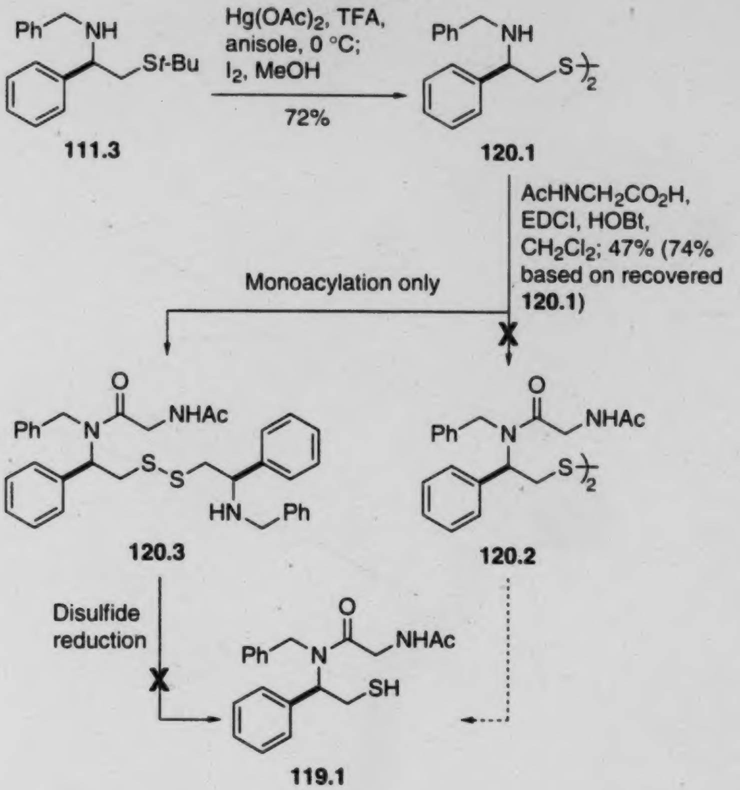
coupled product **119.1** in 62% yield.

Not surprisingly, compound **119.1** exists as a rotamer mixture at room temperature and, consequently, has a rather complex ^1H NMR spectrum. Additionally, the compound exhibits some unusual chemical shift values for certain of its protons. In particular, there are several multiplets at about 4.6 to 5.8 ppm, whereas neither of the starting materials shows any signals above approximately 4.1 ppm except, of course, the aromatic and amide-NH signals, which the present multiplets did not represent. As a result of these unusual chemical shift values, it was initially difficult to establish with certainty that the desired coupled product **119.1** had indeed formed, and so we had to resort to the preparation of structurally related compounds. We were able to establish at least, that our presumed compound **119.1** was likely in the form of a thiol and not a disulfide, on the basis of both mass spectral data and a D_2O ^1H NMR exchange experiment (the pair of triplets at 1.39 and 1.29 ppm disappeared on addition of D_2O).

We first attempted to prepare the expected coupled product (**119.1**) by an independent route so that we would have reference spectra of authentic material, but we were not successful. Compound **111.3** was subjected to standard thiol deprotection conditions, and then treated with I_2 to form disulfide **120.1** (Scheme 120). Attempted acylation of this species, however, gave only the monoacylated product **120.3**, and not the desired compound **120.2**. Interestingly, compound **120.3** also displayed very similar ^1H NMR signals in the range 4.6 to 5.8 ppm to what had been observed with our presumed coupled product **119.1**. Attempted reduction of the disulfide bond of the monoacylated compound **120.3**, in order to obtain **119.1**, resulted in the formation of a complex mixture.

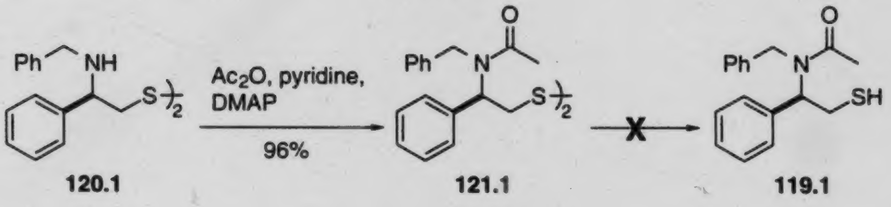
We next prepared the even simpler diacylated compound **121.1** from disulfide **120.1** (Scheme 121). This experiment was successful, and gave material which again displayed the unusual chemical shift values we had observed previously. However, we were not able to cleave the disulfide bond to generate the free thiol.

Scheme 120


Hg(OAc)2, TFA, anisole, 0 °C; I2, MeOH
72%
111.3
120.1
AcHNCH2CO2H, EDCI, HOBt, CH2Cl2; 47% (74% based on recovered 120.1)
Monoacylation only
120.3
120.2
Disulfide reduction
119.1

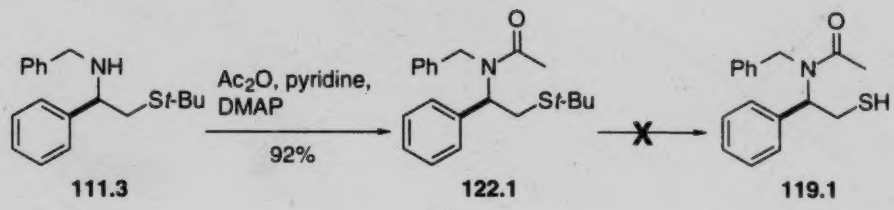
Finally, compound **111.3** was acylated under the conditions shown in Scheme 122, and gave thioether **122.1**. The ^{1}H NMR spectrum of this material was very informative and we were able to assign all of its proton signals for each of the rotamers. By reference to this spectrum, we were now able to assign the signals of all of the protons for each of the rotamers in our coupled product **119.1** (Scheme 119), thereby confirming

Scheme 121



its structure. We were not, however, able to deprotect the thioether **122.1**, although we used a variety of conditions. We previously had found that deprotection could be carried out when the adjacent nitrogen is not acylated.

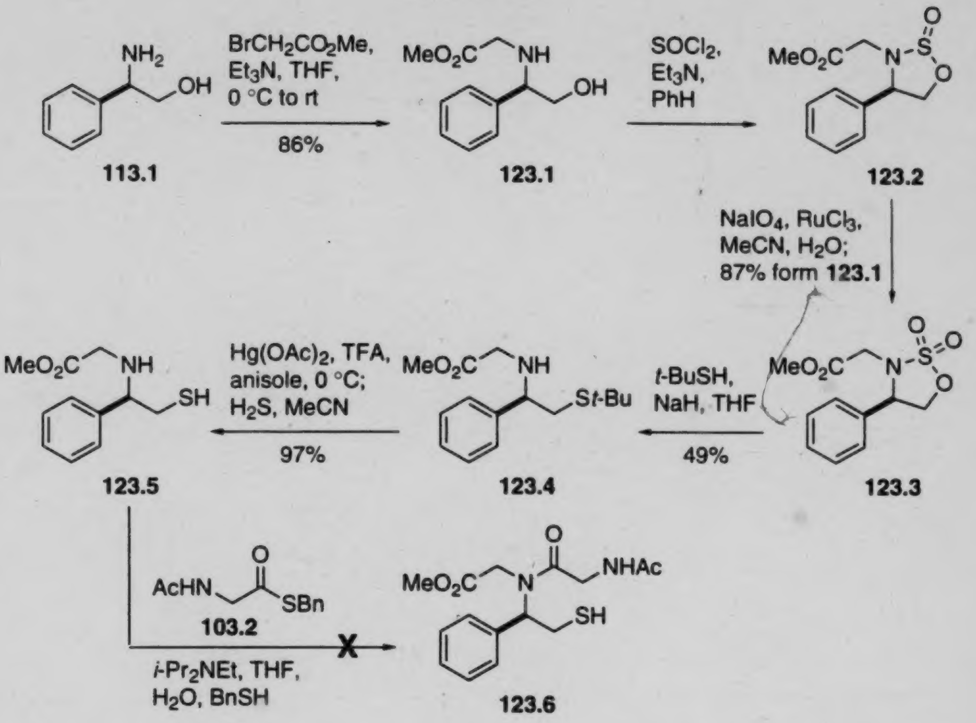
Scheme 122



We next turned our attention to extending the successful coupling approach just described (see Scheme 119) to a slightly more complicated tether-bearing system, obtained from an actual amino acid derivative. The preparation of the tethered compound followed our earlier successful approach, and is outlined in Scheme 123. (*R*)-Phenylglycinol (**113.1**) was *N*-alkylated using methyl 2-bromoacetate,[107] to give amino acid ester **123.1**. Although we were repeating a literature procedure, the preparation of **123.1** was initially troublesome in that a low yield of the desired product was obtained, along with byproduct formation. Although the bromide that we used for the alkylation appeared to be of high quality, as judged by its spectral data, we found that when a fresh bottle of the bromide was used, we could repeat the alkylation reaction exactly as described in the literature.

Compound **123.1** was treated with $SOCl_2$ and Et_3N to give cyclic material **123.2**, which was then oxidized to **123.3** in high overall yield. Alkylation of *t*-BuSH was then conducted using **123.3**, but we consistently obtained a low yield of the desired product **123.4**, under a variety of conditions. The thioether (**123.4**) so obtained was then treated under standard conditions to remove the thiol protecting group and liberate thiol **123.5**. This species was then used in an attempted coupling reaction with thioester **103.2** to give

Scheme 123

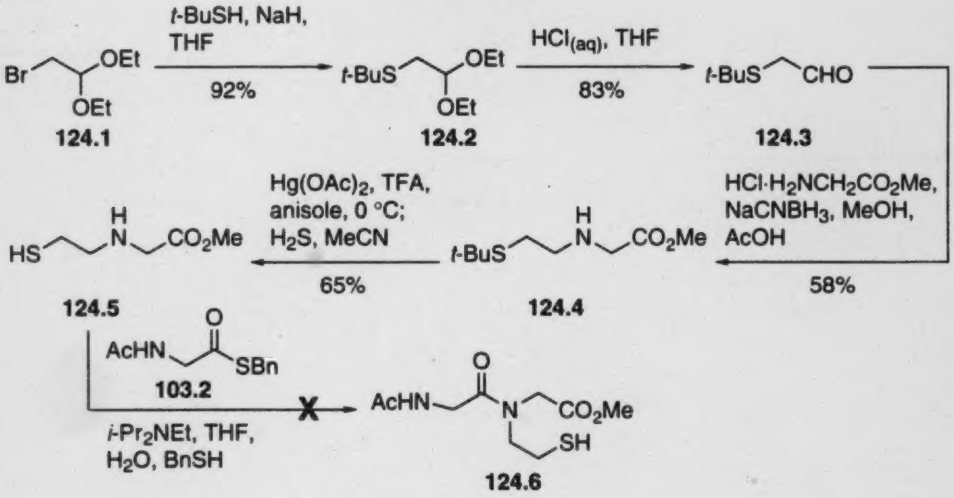


123.6, but we were not able to effect the desired reaction.

We had begun to suspect that the reason we were unable to use our amino acid derivative **123.5** in a ligation/acyl transfer reaction, might have more to do with the actual ligation conditions than the substrate itself. After all, the coupling reaction was easily conducted using the related tether-bearing amine **110.1**. All of the coupling reactions that have been described by Kent have been conducted in an aqueous phosphate buffer, using fully deprotected water-soluble peptides, and these conditions differ significantly from those we used with **123.5**. We therefore decided to prepare an analog of a system that had already been investigated by Kent (*cf.* **54.5a**, Scheme 54), and which had been shown to be effective in the ligation and acyl transfer, when his standard coupling conditions were used. We would then attempt ligation of this species using our conditions. An unsuccessful coupling reaction in this case would support the assumption that the observed lack of reactivity between **123.5** and **103.2** was likely due to the ligation conditions, and not the substrate. On the other hand, if coupling did occur, then this would suggest that the tether we were examining was not as amenable to the coupling process as we had hoped.

The test compound we prepared is thioamine **124.5** (Scheme 124). Its preparation began with *S*-alkylation of *t*-BuSH using bromoacetal **124.1**. Acetal hydrolysis gave the corresponding aldehyde (**124.3**), which was subsequently converted into thioether **124.4** under standard conditions. Thiol deprotection then gave the required thioamine (**124.5**). Attempted coupling between **124.5** and thioester **103.2** under the conditions shown, did not result in any of the desired coupled material (**124.6**). Hence, it appears that, for reasons which are unclear, it is important that the coupling reaction be conducted using Kent's conditions.

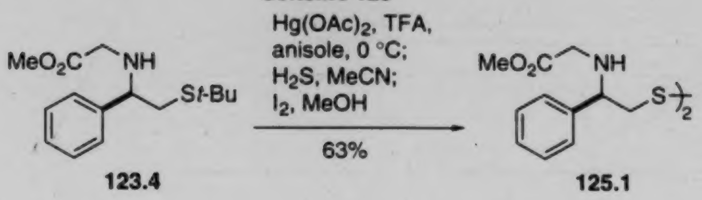
Scheme 124



Therefore we set out to develop conditions similar to Kent's which would favor coupling between **123.5** and **103.2**. In our case we could not use Kent's exact conditions because our materials were not water-soluble. Although we did spend a considerable amount of time preparing several compounds related to **123.5** and **103.2**, but which were somewhat more water soluble, we preferred to use compounds such as **123.5** and **103.2** because they would provide the ligation/acyl transfer product in a form that was amenable to isolation using standard extraction procedures and purification by silica gel chromatography. Isolation and purification using more polar versions of **123.5** and **103.2** would be much more involved. After a considerable amount of exploratory work, we found that we could conduct the ligation/acyl transfer experiments using compounds **123.5** and **103.2** in a mixture of aqueous phosphate buffer and a water-miscible organic solvent. As a result of these exploratory studies, a few general features of the coupling reaction became clear, and these are described below.

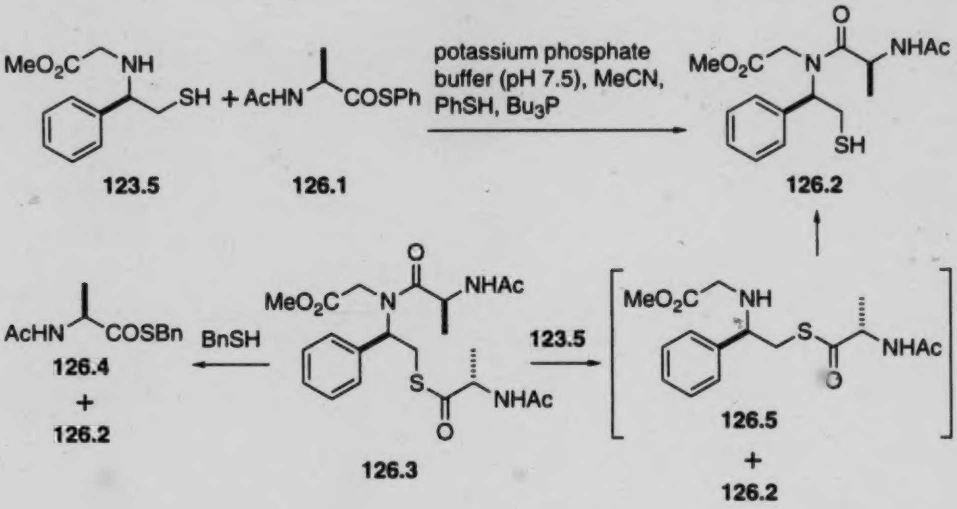
Our initial studies on the development of suitable coupling conditions for our model reactions involved the use of thioamine **123.5**, and it soon became apparent that when this thiol was used, competing disulfide formation occurred prior to the ligation, thus preventing ligation from going to completion. For instance, **123.5** was allowed to react with **103.2** in MeCN and sodium phosphate buffer (pH 7.5), in the presence of BnSH and PhSH, which gave the desired product, but in only 42% yield. The course of the reaction was monitored by HPLC, and this showed that after about 12 h, there was no further consumption of the thioester. Disulfide formation was occurring even though the additives PhSH and BnSH — which are supposed to prevent disulfide formation (see Section 1.5.1a) — were being used. Even on its own, **123.5** has a tendency to form the corresponding symmetrical disulfide. Fortunately, we found that disulfide formation could be prevented (*cf.* Section 1.6.2) in our system if the reaction was carried out in the presence of either Bu_3P or $(HO_2CCH_2CH_2)_3P{\cdot}HCl$.[108] Furthermore, in order to avoid problems introduced by the tendency of thiol **123.5** to form the disulfide, we deliberately converted it into the symmetrical disulfide **125.1** (Scheme 125). This was then used in the ligation/acyl transfer process by first incubating it for a short time with the phosphine prior to addition of the thioester. Thus, the oxidation-sensitive thiol was generated *in situ* from the corresponding disulfide.

Scheme 125



We also established that it was not sufficient to use only the thiol additive PhSH in the reaction mixture, and that it was essential that BnSH also be present (*cf.* Section 1.5.1a). When coupling between **123.5** and **126.1** (Scheme 126) was carried out in the presence of PhSH, but without any BnSH present, the desired product (**126.2**) was obtained along with thioester **126.3**. When BnSH was present, no products corresponding to thioester **126.3** were ever observed. Presumably, in the absence of BnSH, the coupled material (**126.2**) was simply undergoing thioester exchange with additional **126.1**, and that this equilibrium strongly favored formation of **126.3**. However, when the more nucleophilic BnSH was present, it reacted with any species such as **126.3** that might have formed, regenerating the desired product (**126.2**) as well as the benzyl thioester **126.4** which could then undergo ligation.

Scheme 126



In the context of thiol nucleophilicity (see Section 2.2.2b), the fact that **126.3** was isolated from a reaction mixture containing PhSH is significant, as it suggests that the thiol group of benzylic tether **123.5** is more nucleophilic than PhSH. This, in turn, implies that, as suggested above, the benzylic tether **80.2** (Scheme 80) is a better nucleophile than aromatic thiol-containing tether **80.1**, and so is potentially better suited as a coupling device. Of course, it could be argued that if the tether in question is strongly nucleophilic, then **123.5** should have been able to undergo ligation (Scheme 126) with thioester **126.3** to give the required acyl transfer intermediate (**126.5**) and, ultimately, the desired product (**126.2**). The fact that this reaction did not appear to occur, however, may simply be due to the increased steric bulk of **123.5** as compared to BnSH.

We also established during the course of these experiments that phenyl and benzyl thioesters could be used interchangeably without effecting the outcome of the reaction, as long as BnSH was present in the reaction mixture. When a phenyl thioester was used, it underwent rapid (less than 30 min) thioester exchange with BnSH, generating the benzyl thioester *in situ*. After a reaction period of 30 min, we detected no phenyl thioester in the reaction mixture, and so we assume that it is the benzyl thioester that is involved in the subsequent ligation. However, Kent has stated (see Section 1.5.1a) that when a benzyl thioester is used in coupling reactions in the presence of added PhSH, thioester exchange occurs to give the corresponding phenyl thioester, and that it is this species that is involved in the ligation. Although our own observations do not seem to support this claim, we arbitrarily chose to continue to use PhSH as a thiol additive (as well as BnSH) for our preliminary investigations.

We next examined what effect the relative amount of buffer and organic solvent would have on the rate of coupling for the formation of **123.6**, and determined that there is a strong dependency of the rate on the solvent composition. The half-time values shown in Table 16 were observed for the reaction between either thiol **123.5** or disulfide **125.1** and benzyl thioester **103.2** (or the corresponding phenyl thioester **127.1**, Scheme 127) to give **123.6**. The reactions were monitored by HPLC, and the half-times for the reactions were determined by inspection of the HPLC traces. These reactions were conducted at 37 °C, in the presence of Bu_3P, BnSH and PhSH, and at approximately 100 mM concentration with respect to the substrates. The experiments corresponding to the first three entries of Table 16 were carried out using MeCN as the organic solvent. However, due to solubility problems, MeCN was not useful beyond 2:1 MeCN to buffer, and so we changed to DMF, which avoided the solubility problems. The data in Table 16 clearly show that the rate of reaction is strongly dependent on the proportion of phosphate buffer used. In each case, after an extended reaction time, a small amount of material was observed which, on the basis of experiments that will be described later, we believe to be acid **127.2** (Scheme 127).

Table 16

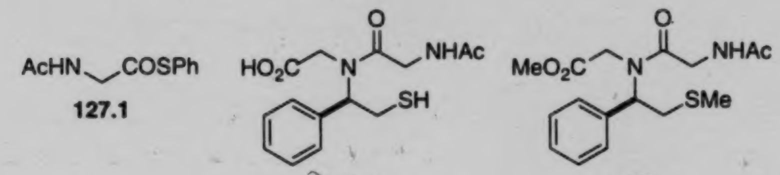
Conditions Used and Half-times Observed for Model Coupling Reactions for the Formation of **123.6** Conducted at Approximately 100 mM Concentration[*]

Solvent System	Tether-bearing Amino Acid Component	Thioester	Coupling Reaction Half-time (approximate)
MeCN-buffer[†] (4:1)	**123.5**	**127.1**	24 h
MeCN-buffer (2:1)	**123.5**	**127.1**	7 h
MeCN-buffer (2:1)	**125.1**	**127.1**	5 h
DMF-buffer (1:1)	**125.1**	**127.1**	1 h
DMF-buffer (1:2)	**125.1**	**103.2**	45 min

[*] All reactions were conducted at 37 °C in the presence of Bu_3P, BnSH, PhSH

[†] Sodium phosphate buffer, pH 7.5

Scheme 127



127.1 **127.2** **127.3**

We also conducted a similar set of experiments at a concentration of approximately 10 mM, so that we could more directly compare our data with those reported in the literature for other coupling systems. Interestingly, we observed very little change in the half-time of the reaction, even though we had decreased the concentration 10 fold. In fact, by simply increasing slightly the amount of phosphate buffer relative to DMF, we could obtain coupling rates comparable to our best results at the higher concentration of 100 mM. Unfortunately, although the coupling reactions did proceed at a reasonable rate at the lower concentration of 10 mM, ester hydrolysis to afford **127.2** was a significant side reaction, and in all cases the desired coupled product was essentially completely hydrolyzed with in 22 h.

The fact that the hydrolysis product had formed was determined in the following way. After the reaction was complete, the mixture was acidified and then extracted with CH_2Cl_2. ^1H NMR analysis of the material obtained by evaporation of the organic extract revealed that the methyl signals of the methyl ester had disappeared. When this material was treated with CH_2N_2 and the crude product examined by ^1H NMR, the methyl signals of the methyl ester were observed, but the compound we were now detecting was actually thioether **127.3** (Scheme 127): besides restoring the methyl ester, CH_2N_2 had also methylated the thiol.

Table 17

Conditions Used and Half-times Observed for Model Coupling Reactions for the Formation of **123.6** Conducted at Approximately 10 mM Concentration*

Solvent System	Tether-bearing Amino Acid Component	Thioester	Coupling Reaction Half-time (approximate)
DMF-buffer† (1:2)	**125.1**	**103.2**	1 h
DMF-buffer (1:3)	**125.1**	**103.2**	30 min
DMF-buffer (1:3)	**125.1**	**103.2**	30 min

*All reactions were conducted at 37 °C in the presence of BnSH and PhSH. Reactions corresponding to the first two entries were conducted using Bu_3P. Reaction corresponding to the third entry was conducted using $(HO_2CCH_2CH_2)_3P{\cdot}HCl$.

†Sodium phosphate buffer, pH 7.5

The work that has been described up to this point represents the present state of the research that we have been conducting using benzylic tether **80.2**. As a result of the studies, we have been able to establish that the tether is suitable as a coupling device in our planned approach to segment coupling. The problem of ester hydrolysis that we observed in our model system is not likely to be significant in the overall context of our coupling approach, because the components that we will eventually be using for coupling will be unprotected peptides, and so will not posses any easily hydrolyzable substituents. We will, however, have to prepare a new model system for further studies that has an amide in place of the methyl ester, to avoid hydrolysis. By appropriate adjustment of the coupling conditions, we were able to obtain a rate for the coupling reaction that is comparable to values observed by Kent (see Section 1.5.2) and Dawson[100] in their systems. Based on the correlation between reaction rate and the relative amount of phosphate buffer that we observed, it seems reasonable to assume that, once our system is applied to fully unprotected peptide segments using only phosphate buffer and no organic solvent, the reaction rates will increase further.

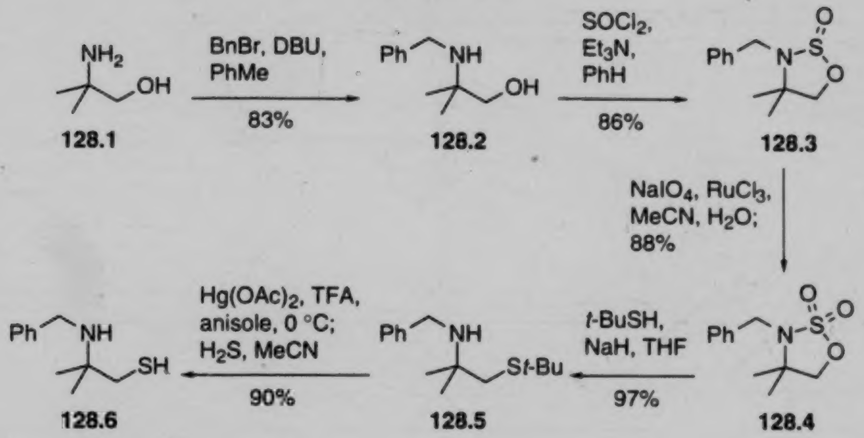
2.2.5 Studies Involving Dimethyl Tether 80.3

For the last part of this section on segment coupling involving ligation by thioester exchange, our preliminary investigations into the use of dimethyl tether **80.3** will be described.

(a) Synthesis of a Model System for Ligation and Acyl Transfer

The model system that we used for our initial investigations with dimethyl tether **80.3** is compound **128.6**, which was prepared as shown in Scheme 128, using a similar route to the one we had used previously. Amino alcohol **128.1** was first alkylated by treatment with BnBr and DBU to give secondary amine **128.2**, which was then treated with $SOCl_2$ in the presence of base to afford **128.3**. This was then oxidized to compound **128.4**, which was subsequently used to alkylate *t*-BuSH, giving **128.5**. The alkylated

Scheme 128

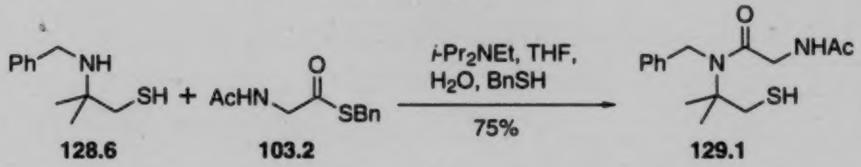


product so obtained was subjected to our standard deprotection protocol, affording the required thioamine (**128.6**) in very good yield.

(b) *Ligation and Acyl Transfer*

With compound **128.6** in hand, we next examined its use for ligation and acyl transfer. To do this, **128.6** was combined with thioester **103.2** under the conditions shown (Scheme 129), a procedure which gave the desired product (**129.1**) in good yield. The coupling reaction in this case was very slow, taking approximately 39 h. The related reaction described above for compound **110.1** (Scheme 119) took about 11 h. The experiment shown in Scheme 129 was conducted prior to the development of the improved coupling conditions described above at the end of Section 2.2.4b, and we have not yet tried coupling **128.6** and **103.2** under the new conditions. Clearly, however, dimethyl tether

Scheme 129



80.3 does show promise for use as a segment coupling device.

2.3 Segment Coupling Approaches Involving Nucleophilic Acylation Catalyst Derivatives

While our work on segment coupling approaches involving ligation by thioester exchange (see Section 2.2) was being conducted, we began a brief investigation into some ideas we had regarding the development of a new method for achieving prior ligation of peptide segments. The reactions we had in mind were based largely on known chemistry related to nucleophilic acylation catalysis, but the overall approach is quite similar to the work described above.

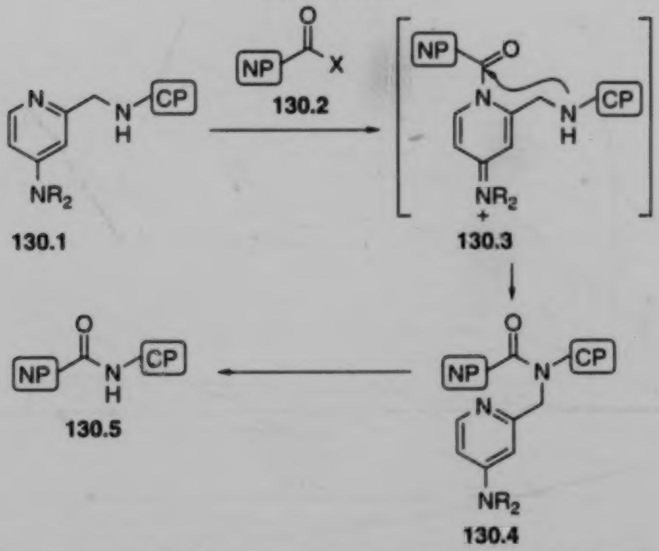
2.3.1 Proposed Research

In our planned approach to a new ligation method, instead of relying on nucleophilic attack by a thiol substituent on a thioester as a means of ligating the peptide segments prior to acyl transfer, we thought that it might be possible to replace the thiol with a different nucleophile. Of course, we might also have to replace the thioester with a different acylating component, in order to make the two species compatible. The new nucleophile would have to be powerful so that it could overcome the barriers associated with bringing two peptide segments together in solution (see Section 1.2), and we reasoned that a good choice for the nucleophilic component would be one that was well know to function as an acylation catalyst in intermolecular coupling reactions.

Acylation catalysis has been the subject of a considerable amount of investigation, and several excellent nucleophilic catalysts have been developed, including compounds such as DMAP (and related species), imidazole, and HOBt. In general, in an acylation involving one of these catalysts, the acylating component undergoes initial attack by the nucleophilic catalyst, giving rise to an activated intermediate. This intermediate, in turn, is attacked by a second, but less reactive nucleophile, such as an amine, which is ultimately acylated.

Our planned approach to segment coupling using this new type of ligation strategy is outlined in a general way by reference to the DMAP-based system shown in Scheme 130. Just as in the previous approaches (see Section 2.2), we planned to prepare special tether-bearing amino acid derivatives (*cf.* **130.1**). The tether in this case would have incorporated into it the nucleophilic catalyst. The catalyst portion of this species would be allowed to react with a suitably activated acyl component (**130.2**), giving the activated acyl transfer intermediate **130.3**. Peptide bond-forming intramolecular acyl transfer from this species would give the coupled product **130.4**, and all that would remain at this point would be removal of the tether (**130.4** → **130.5**).

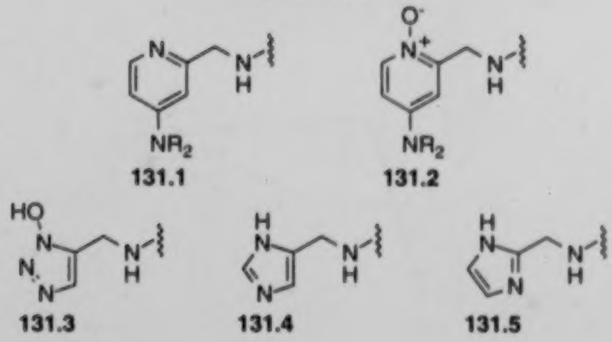
Scheme 130



NP = *N*-terminal peptide; CP = *C*-terminal peptide; X = leaving group

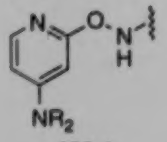
Several catalyst-bearing tethers were considered, and these are shown in Scheme 131. Systems **131.1** and **131.2** are based on DMAP-like catalysts, **131.3** is based on HOBt, and **131.4** and **131.5** are, of course, based on imidazole. In all cases, the

Scheme 131



systems are benzylic-like amines, and so could potentially be removed, following coupling, once they are in the form of benzylic-like amides, by exposure to strong acid, just as we had planned to do for the benzylic tethers **80.1** and **80.2** (Scheme 80). Alternatively, the tethers could be attached to the amino group of the amino acid component by an N-O bond (*cf.* **132.1**, Scheme 132), and so potentially they should be removable by reductive cleavage of that bond, following coupling. Use of an N-O linked system might also confer increased nucleophilicity to the amine, via the alpha effect, thus making the intramolecular acyl transfer reaction more efficient. The question of the most appropriate form for the acylating component is more difficult to predict, and would likely involve some experimentation. One can imagine that a thioester might be suitable, as might an appropriately activated thioacid (*cf.* Section 1.6.5), or carboxylic acid.

Scheme 132



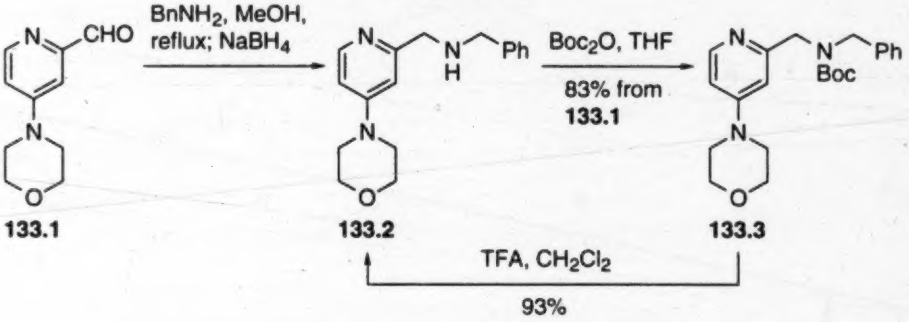
132.1

2.3.2 *Studies Involving DMAP-based Tether 131.1*

(a) Synthesis of a Model System for Ligation and Acyl Transfer

In an effort to determine whether or not the above approach would be useful as a means of ligating peptide segments, we decided to prepare the DMAP-like system **133.2** (Scheme 133). This compound was generated from the known aldehyde **133.1** and $BnNH_2$, using our standard conditions for imine formation and reduction. Due to the extremely polar nature of **133.2**, it could not be purified by silica gel chromatography, and so the crude material was treated with Boc_2O to give compound **133.3**, which could now be purified chromatographically. The desired compound (**133.2**) could then be generated in pure form by treating **133.3** with TFA.

Scheme 133

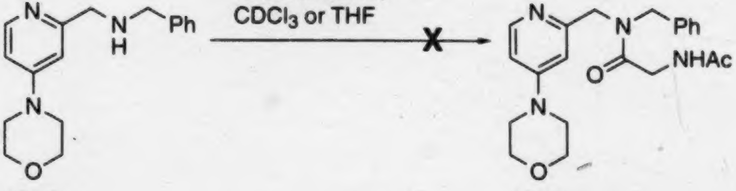


(b) *Ligation and Acyl Transfer*

With the required model system in hand, we began to explore whether or not it could function in the ligation and acyl transfer process. Our first attempts at effecting ligation between **133.2** and an acyl component involved the use of thioesters **103.2** and **127.1**. Equimolar amounts of **133.2** and either of the two thioesters were combined in either $CDCl_3$ or THF (Scheme 134) but, even after 12 h, there was no indication that any reaction had occurred. This observation suggested that either the catalyst-containing tether was not nucleophilic enough, or that the thioesters were not sufficiently activated.

For DMAP-like systems, a potentially more nucleophilic version might be obtained by using the pyrrolidine-substituted pyridine **135.1**. 4-(Pyrrolidino)pyridine is known to

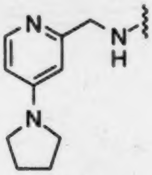
Scheme 134



133.2 **134.1**

be slightly superior to DMAP as an acylation catalyst, but is 10 times more nucleophilic than 4-(morpholino)pyridine.[109] Increased nucleophilicity in the tether might also be achieved by switching to one of the other systems shown in Scheme 131.

Scheme 135

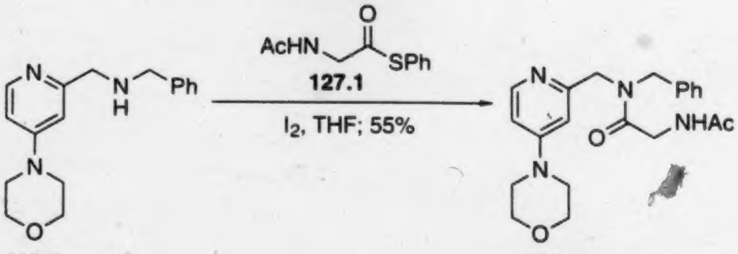


135.1

In an effort to activate thioesters to attack by compound **133.2**, the ligation was conducted in the presence of $AgNO_3$, in the hope that the highly thiophilic silver ion would coordinate the sulfur and make it a better leaving group (*cf.* **58.3** → **58.5**, Scheme 58). However, when this reaction was carried out, all that was obtained, using various solvent systems, was an insoluble precipitate. Presumably the pyridine subunit was irreversibly complexing the silver ion, forming the precipitate. In order to avoid this unwanted complexation, we attempt to activate the sulfur by treatment with I_2. Under these conditions, **133.2** and thioester **127.1** did react to form the desired product (Scheme 136), albeit in modest yield.

This experiment is the last done in our investigations into the new approach to segment ligation. It is much too early to know if the systems shown in Scheme 131 will be

Scheme 136



133.2 **134.1**

suitable as coupling devices. We will have to investigate alternative activated acyl components, and explore the other tethers shown in Scheme 131. We also still have to establish if, indeed, the coupling we observed has occurred as a result of prior ligation and intramolecular acyl transfer, or if it is simply due to an intermolecular process.

3 CONCLUSION

Although a considerable amount of work remains to be done before we are able to achieve all the objectives outlined in this Chapter, several have already been realized. We were able to develop a synthetic route to compounds possessing benzylic tether **80.1**, and establish that the tether works as a coupling device. We also acquired support for the assumption that coupling using this tether does indeed appear to operate by a prior ligation and intramolecular acyl transfer mechanism, and is not simply the result of intermolecular acylation. Furthermore, by examining benzylic tether **80.1**, we gained some important information regarding the extension of our proposed approaches, so that they might be useful in an iterative context. While we would have preferred, of course, that the work reported by Dawson had not, in fact, been done in another laboratory, as it turned out, tether **80.1** is far from ideal. As well, much of what we learned while examining this tether is applicable to the other systems we are interested in, and so the work is still of value to us.

When the focus of our research shifted to tether **80.2**, once we had established a synthetic route to systems based on it, and had begun to use HPLC as a tool to monitor the course of the ligation reactions, we began to make rapid progress towards establishing suitable conditions for carrying out model coupling reactions. The observations will be of great value as we begin to examine model systems derived from various amino acids, in a effort to determine the generality of our approach.

Finally, our brief look at the use of dimethyl tether **80.3** as a coupling tool suggested that the system is worthy of future study. As well, the quick investigation that we undertook regarding our ideas on a new ligation strategy based on acylation catalysis, has suggested that continued research in this area is also justified.

4 FUTURE RESEARCH

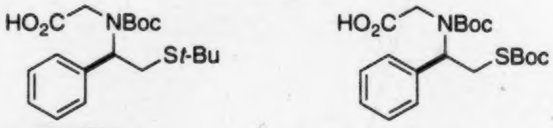
4.1 Segment Coupling Approaches Involving Ligation by Thioester Exchange

As a result of the work described in Section 2.2, the ability of the three tethers depicted in Scheme 80 to function as coupling devices has been established. Given the similarity of our approach involving tether **80.1** to that reported by Dawson,[100] pursuit of our research using this tether is not strongly warranted at present. However, continued investigation into segment coupling using tether **80.2** and **80.3** is justified, given that the problem of developing a generalized method to segment coupling has not been solved.

The data summarized in Tables 16 and 17 were obtained from experiments aimed at establishing conditions that would allow coupling to be carried out between model tethered system **123.5** or **125.1** and thioester **103.2** or **127.1**. As a result of these experiments, it was found that the coupling reaction can be made to proceed in an acceptable manner when carried out at 10 mM concentration, using a 1:3 mixture of DMF to phosphate buffer, in the presence of a phosphine and thiol additives. However, competing hydrolysis to afford the carboxylic acid **127.2** was a significant side reaction under these conditions. As mentioned earlier, this fact is not likely to be significant in the overall context of our approach to segment coupling, since we ultimately intend to use fully unprotected peptide segments which, of course, do not posses any easily hydrolyzable substituents. However, for the purpose of establishing whether or not our coupling technique is general, we will have to prepare systems related to **125.1**, but which have an amide group in place of the methyl ester. Presumably these systems will not undergo the hydrolysis side reaction. If this is indeed the case, then we will be in a position to prepare other tethered systems from other amino acids. We will also prepare other thioesters — also from other amino acids — and then try the coupling reactions using various combinations of these. As mentioned in Section 1.4.2b, it has been argued[34,50] that, for unassociated peptide segments, the rate of peptide bond formation is largely determined by the two substituents that neighbor the new bond. Hence, these model coupling reactions in which relatively simple dipeptides are formed, should provide a reasonable representation of reactions in which actual segments will be coupled. If we are indeed successful in establishing that our approach is generally applicable, then the following short-term objectives will be pursued.

The first matter that will require attention is the preparation of a derivative of **123.4**, in which the methyl ester is replaced by a carboxyl group and the nitrogen is Boc protected (**137.1**, Scheme 137), and we will have to demonstrate that we can use this special amino acid residue to cap the *N*-terminus of the *C*-terminal peptide, in the last step of its synthesis (*cf.* **71.1** + **71.2** → **71.3**, Scheme 71). Although we ultimately intend to conduct the capping reaction on the solid phase, for the present purposes, it should suffice to simply demonstrate this process in solution through the formation of a di- or tripeptide. Preparation of the required amino acid derivative (**137.1**) should be possible from **123.4** in a single step, by treating it with Boc_2O in aqueous NaOH and THF. Once capping of the *C*-terminal segment has been achieved, then the resulting peptide will be subjected to our standard deprotection conditions, in order to show that we can liberate the thiol and amino groups, and the product will then be used in a ligation/acyl transfer reaction.

Scheme 137



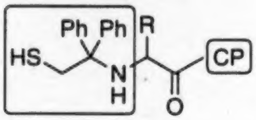
137.1 **137.2**

When it has been established that the *C*-terminal peptide can be capped in the required way, and that the resulting system can be deprotected and used in a coupling reaction, we will then be in a position to begin to prepare larger (tri- or tetrapeptide) model segments (both the *C*- and *N*-terminal peptides), which should be fully water soluble, and we can then investigate the coupling in an aqueous environment — that is, with out the addition of DMF as a co-solvent. If the trend that was observed in Tables 16 and 17 continues, then we should see even faster rates for the coupling reaction than we have observed up to this point. Prior to embarking on the synthesis of the water soluble segments, however, it will likely be advantageous to develop a preparation of *S*-*N*-bisBoc-protected tethered amino acid derivatives (*cf.* **137.2**). Use of these systems in place of the *S*-*t*-Bu, *N*-Boc compounds (*cf.* **137.1**) will allow for an easier deprotection sequence.

Another matter that will require prompt attention is cleavage of the tether from the coupled product. This is easily examined by subjecting the product of the coupling reaction to a strong acid such as TFA or HF. Of course, as was mention in Section 2.2.2a, if it turns out that the tether is not labile under these conditions, then systems which have a *para* -substituted electron releasing group, such as a methoxy substituent, will need to be prepared and examined.

In the long-term, several other matters will require attention. We will have to examine systems in which the tether is derived from *S*-, rather than *R*-phenylglycinol to establish if, indeed, there is a significant difference in coupling rates using the diastereomeric *C*-terminal segment (see Section 2.2.2c). Related to this, is the preparation and examination of a diphenyl substituted tether (**138.1**, Scheme 138), whose structure avoids the issue of diastereoisomerism altogether. The dimethyl tether **80.3** will also need to be examined in much the same way as **80.2**. Of course, if our segment coupling approach proves to be successful as a generalized method, then we will begin to investigate matters related to the extension of the technique so that it can be used in an iterative sense, and then in a solid phase procedure, as described in Sections 2.2.1b and 2.2.1c.

Scheme 138



138.1

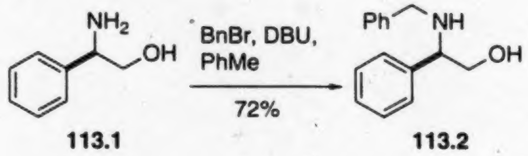
4.2 Segment Coupling Approaches Involving Nucleophilic Acylation Catalyst Derivatives

Relative to the thioester exchange approach to ligation discussed above, we have not conducted much experimental work on ligation involving nucleophilic acylation catalyst derivatives. Hence, it is difficult to suggest in detail what is required in terms of future investigation. Initial work in this area will likely have to be approximately equally divided between the preparation of the other tethers shown in Scheme 131, and the development of conditions for coupling that work better than the iodine-mediate conditions shown in Scheme 136. If, as a result of this study, we are able to develop a system (or systems) and conditions that are suitable for coupling, then we will follow a course of research much like that described above for the thioester exchange-based ligation strategy, the ultimate objective being to apply the ligation technique to the coupling of peptide segments.

5 EXPERIMENTAL

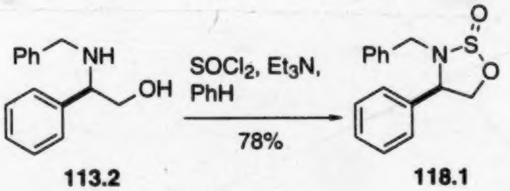
The same general procedures that were described in Chapter 1 apply.

N-(Benzyl)-(*R*)-phenylglycinol (113.2)



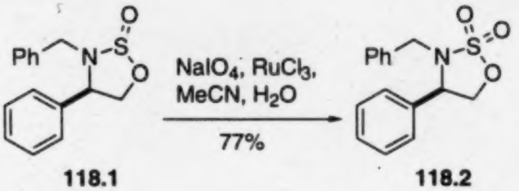
BnBr (4.30 mL, 36.11 mmol) was added by syringe pump over *ca*. 30 min to a stirred suspension of (*R*)-phenylglycinol (4.954 g, 36.11 mmol) and DBU (5.40 mL, 36.1 mmol) in dry PhMe (100 mL) (N_2 atmosphere). The mixture was stirred for 1 h after the addition, and then H_2O (100 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (6 x 25 cm), using 80:20 EtOAc-hexanes, gave **113.2** (5.946 g, 72%) as a pure (^1H NMR DMC XV-139-A) white solid. The ^1H NMR data were the same as those reported previously.[102]

3-Benzyl-4-(*R*)-phenyl-1,2,3-oxathiazolidine-2-oxide (118.1)



A solution of $SOCl_2$ (0.31 mL, 4.3 mmol) in dry PhH (5 mL) was added over *ca*. 10 min to a stirred and cooled (ice-water bath) solution of **113.2** (0.912 g, 4.03 mmol) and Et_3N (1.24 mL, 8.90 mmol) in dry PhH (20 mL) (N_2 atmosphere). The cooling bath was removed and the mixture was stirred for 1 h and then washed with H_2O (3 x 10 mL) and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 25 cm), using 40:60 EtOAc-hexanes, gave the faster eluting diastereomer of **118.1** (contaminated with a trace of the slower eluting diastereomer) as a white solid (^{1}H NMR DMC XV-77-A) and the slower eluting diastereomer of **118.1** (contaminated with a trace of the faster eluting diastereomer) as a white solid (^{1}H NMR DMC XV-77-B). The material was combined to give a white solid (0.8593 g, 78%) that was used as such in the following transformation. The faster eluting diastereomer had: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 3.91 (d, J = 16.0 Hz, 1 H), 4.37-4.52 (m, 2 H), 4.64-4.79 (m, 2 H), 7.08-7.19 (m, 2 H), 7.24-7.48 (m, 8 H). The slower eluting diastereomer had: ^{1}H NMR (CDCl$_3$, 360 MHz) δ 3.92-4.03 (m, 2 H), 4.24-4.33 (m, 1 H), 4.67-4.78 (m, 1 H), 4.98-5.08 (m, 1 H), 7.23-7.52 (m, 10 H).

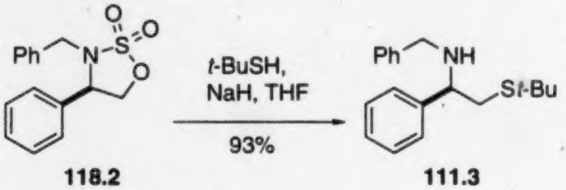
3-Benzyl-4-(*R*)-phenyl-1,2,3-oxathiazolidine-2,2-dioxide (118.2)



RuCl$_3$·3H$_2$O (*ca.* 5 mg) and NaIO$_4$ (0.8961 g, 4.19 mmol) were added to a stirred and cooled (ice-water bath) solution of **118.1** (0.7602 g, 2.78 mmol) in MeCN (10 mL), followed by H$_2$O (15 mL). The cooling bath was removed and stirring was continued for 1 h, by which time all of the starting material had reacted (TCL control, silica gel, 40:60 EtOAc-hexanes). The mixture was partitioned between EtOAc (75 mL) and H$_2$O (20 mL), and the organic phase was washed with H$_2$O (2 x 25 mL) and saturated aqueous NaCl (20 mL), dried (MgSO$_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 40:60 EtOAc-hexanes, gave **118.2** (0.6195 g, 77%) as a pure (^{1}H NMR DMC XV-179-A) white solid. ^{1}H NMR (CDCl$_3$, 360 MHz) δ 4.11 and 4.27 (ABq,

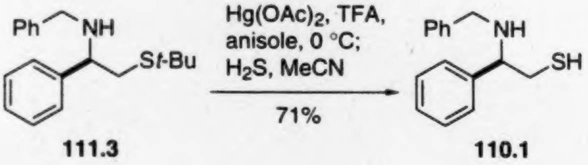
$\Delta\nu_{AB}$ = 60.4 Hz, J = 11.8 Hz, 2 H), 4.35 (t, J = 8.6 Hz, 1 H), 4.63-4.72 (m, 2 H), 7.17-7.28 (m, 5 H), 7.36-7.44 (m, 5 H).

N-Benzyl-(2-*t*-butylsulfanyl)-1-(*R*)-phenylethylamine (111.3)



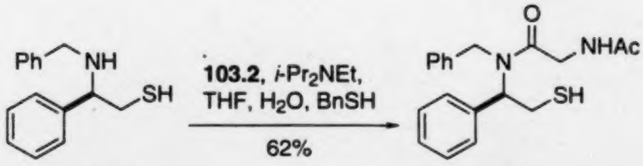
A solution of *t*-BuSH (1.29 mL, 11.44 mmol) in THF (10 mL) was added by cannula over *ca.* 1 min to a stirred suspension of NaH (0.3171 g, 13.21 mmol) in THF (5 mL), with additional THF (2 x 0.5 mL) used as a rinse (N_2 atmosphere). The mixture was stirred for 10 min and a solution of **118.2** (1.6506 g, 5.71 mmol) in THF (3 mL) was added by cannula, with additional THF (2 x 0.5 mL) used as a rinse. The mixture was stirred for 1.5 h, by which time all of the starting material had reacted (TCL control, silica gel, 40:60 EtOAc-hexanes). Aqueous H_2SO_4 (20%) (10 mL) was then added and stirring was continued for 1 h. The mixture was neutralized with saturated aqueous $NaHCO_3$ and combined with EtOAc (50 mL). The organic phase was washed with saturated aqueous NaCl (15 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 40:60 EtOAc-hexanes, gave **111.3** (1.5732 g, 93%) as a pure (^{1}H NMR DMC XVI-39-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.30 (s, 9H), 2.10-2.65 (broad s, 1 H), 2.77 (dd, J = 12.4, 9.7 Hz, 1 H), 2.86 (dd, J = 12.4, 4.3 Hz, 1 H), 3.55 and 3.71 (ABq, $\Delta\nu_{AB}$ = 59.8 Hz, J = 13.4 Hz, 2 H), 3.78 (dd, J = 9.7, 4.3 Hz, 1 H), 7.20-7.45 (m, 10 H).

N-Benzyl-2-(*R*)-phenyl-2-aminoethanethiol (110.1)



A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **111.3** (0.1250 g, 0.42 mmol), followed by $Hg(OAc)_2$ (0.1330 g, 0.42 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (5 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad (1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated to give **110.1** (0.0707 g, 71%) as a pure (^{1}H NMR DMC XV-191-B), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.80-2.10 (broad s, 1 H), 2.73 (dd, *J* = 13.5, 8.1 Hz, 1 H), 2.88 (dd, *J* = 13.5, 5.0 Hz, 1 H), 3.56-3.78 [m, 3 H, including an ABq at δ 3.59 and 3.76 ($\Delta\nu_{AB}$ = 60.0 Hz, *J* = 13.3 Hz)], 7.23-7.47 (m, 10 H).

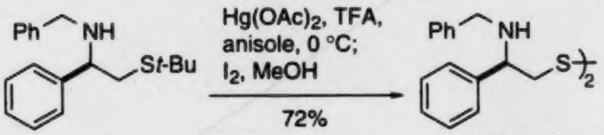
2-[[*N*-(2-Acetamido)acetyl]benzylamino]-2-(*R*)-phenylethanethiol (119.1)



110.1 **119.1**

i-Pr_2NEt (0.10 mL, 0.57 mmol) was added to a stirred mixture of **110.1** (0.0707 g, 0.29 mmol) and **103.2** (0.0653, 0.29 mmol) in THF (3 mL) and H_2O (1 mL), followed by BnSH (*ca.* 0.05 mL). The mixture was stirred for 11 h and combined with EtOAc (25 mL). The organic portion was removed, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1 x 15 cm), using 90:10 EtOAc-hexanes, gave **119.1** (0.0631 g, 62%) as a pure (^{1}H NMR DMC XV-193-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.26-1.44 [m, 1 H, including a t at δ 1.29 (J = 8.6 Hz), and a t at δ 1.39 (J = 8.2 Hz)], 2.00-2.12 (m, 3 H, including a s at δ 2.03 and a s at δ 2.09), 2.85-3.10 (m, 2 H), 3.79-4.98 [m, 4 H, including a d at δ 3.84 (J = 15.3 Hz), a dABq at δ 3.98 and 4.17 ($\Delta\nu_{AB}$ = 57.2 Hz, J = 17.6, 4.2 Hz), an ABq at δ 4.27 and 4.40 ($\Delta\nu_{AB}$ = 45.1 Hz, J = 17.4 Hz) overlapping a dABq at δ 4.46 and 4.60 ($\Delta\nu_{AB}$ = 50.2 Hz, J = 17.4, 3.6 Hz), and a d at δ 4.96 (J = 15.3 Hz)], 5.01-5.72 [m, 1 H, including a dd at δ 5.05 (J = 9.2, 5.6 Hz), and a t at δ 5.67 (J = 7.8 Hz)], 6.56-6.85 (m, 1 H, including a s at δ 6.62 and a s at δ 6.81), 6.97-7.43 (m, 10 H); exact mass (electrospray) *m/z* calcd for $C_{19}H_{22}N_2NaO_2S$ (M + Na) 365.1298, found 365.1295.

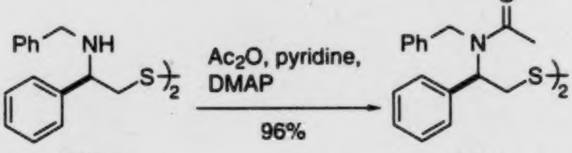
2-(Benzylamino)-2-(*R*)-phenylethyl disulfide (120.1)



111.3 **120.1**

A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **111.3** (0.2852 g, 0.95 mmol), followed by $Hg(OAc)_2$ (0.3035 g, 0.95 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (10 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad (1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (20 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and evaporated to give a residue. The residue was dissolved in MeOH (10 mL) and titrated with saturated methanolic I_2. The resulting mixture was stirred for 10 min and evaporated. Flash chromatography of the residue over silica gel (2.5 x 25 cm), using 80:20 EtOAc-hexanes, gave **120.1** (0.1663 g, 72%) as a pure (^{1}H NMR DMC XVI-57-A), colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.17 (broad s, 2 H), 2.84 (dd, J = 11.2, 8.1 Hz, 2 H), 2.98 (dd, J = 11.2, 4.0 Hz, 2 H), 3.56 and 3.78 (ABq, $\Delta\nu_{AB}$ = 46.7 Hz, J = 12.1 Hz, 4 H), 3.96 (dd, J = 8.1, 4.0 Hz, 2 H), 7.20-7.42 (m, 20 H).

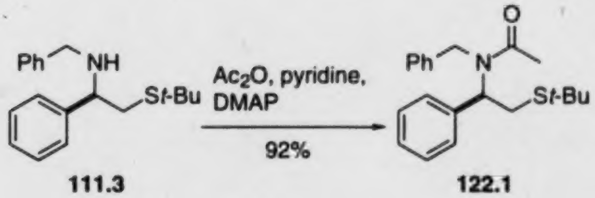
2-[*N*-Acetyl(benzylamino)]-2-(*R*)-phenylethyl disulfide (121.1)



120.1 **121.1**

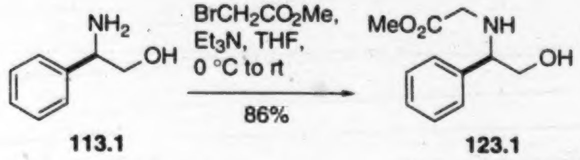
Ac_2O (0.26 mL, 2.79 mmol) was added to a stirred solution of **120.1** (0.0676 g, 0.14 mmol) and DMAP (*ca.* 5 mg) in pyridine (10 mL). The mixture was stirred for 10 h and partitioned between H_2O (10 mL) and CH_2Cl_2 (40 mL). The organic phase was washed with H_2O (2 x 5 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1 x 20 cm), using 80:20 EtOAc-hexanes, gave **121.1** (0.0764 g, 96%) as a pure (^{1}H NMR DMC XVI-75-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.04-2.44 (m, 6 H, including a s at δ 2.08, a s at δ 2.11, a s at δ 2.37, and a s at δ 4.41), 2.88-3.37 (m, 4 H), 3.62-5.05 (m, 4 H), 5.18-5.97 (m, 2 H), 6.95-7.42 (m, 20 H).

N-Benzyl-*N*-[1-phenylethyl-2-(*t*-butylsulfanyl)]acetamide (122.1)



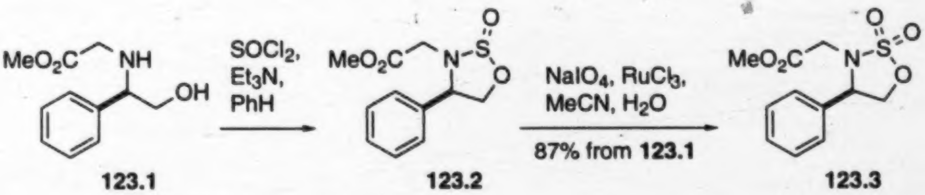
Ac_2O (0.45 mL, 4.76 mmol) was added to a stirred solution of **122.1** (0.7129 g, 2.38 mmol) and DMAP (*ca.* 20 mg) in pyridine (10 mL). The mixture was stirred for 11 h and partitioned between H_2O (10 mL) and CH_2Cl_2 (50 mL). The organic phase was washed with H_2O (2 x 10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 60:40 EtOAc-hexanes, gave **122.1** (0.7481 g, 92%) as a pure (^{1}H NMR DMC XVI-83-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.21-1.32 (m, 9 H, including a s at δ 1.23 and a s at δ 1.31), 2.07-2.47 (m, 3 H, including a s at δ 2.09 and a s at δ 2.44), 2.81-3.09 (m, 2 H), 3.88-4.87 [m, 2 H, including a d at δ 3.93 (J = 15.4 Hz), an ABq at δ 4.26 and 4.41 ($\Delta\nu_{AB}$ = 55.3 Hz, J = 17.7 Hz), and a d at δ 4.83 (J = 15.4 Hz)], 5.12-6.01 [m, 1 H, including a t at δ 5.18 (J = 6.7 Hz), and a t at δ 5.96 (J = 7.9 Hz)], 6.99-7.39 (m, 10 H).

Methyl (*R*)-2-[[*N*-(2-hydroxy-1-phenylethyl)]amino]acetate (123.1)



A solution of methyl 2-bromoacetate (0.76 mL, 8.02 mmol) in dry THF (16 mL) was added dropwise by syringe pump, at a rate of 1 mL per min, to a stirred and cooled (ice-water bath) solution of **113.1** (1.0006 g, 7.29 mmol) and Et_3N (1.22 mL, 8.75 mmol) in dry THF (60 mL) (N_2 atmosphere). The mixture was allowed to warm to room temperature, the cooling bath being left in place for 10 h, but was not recharged during this time, and the mixture was then partitioned between saturated aqueous NH_4Cl (6 mL) and EtOAc (25 mL). The aqueous phase was extracted with EtOAc (2 x 10 mL) and the combined extracts were dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 80:20 EtOAc-hexanes, gave **123.1** (1.3125 g, 86%) as a pure (^{1}H NMR DMC XVII-103-A), colorless oil: The ^{1}H NMR data were the same as those reported previously.[107]

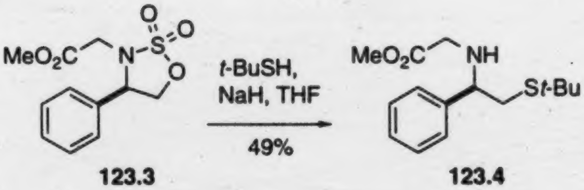
Methyl 2-(4-(*R*)-phenyl-1,2,3-oxathiazolidin-3-yl)acetate S,S-dioxide (123.3)



A solution of $SOCl_2$ (0.40 mL, 5.48 mmol) in dry PhH (5 mL) was added over *ca.*

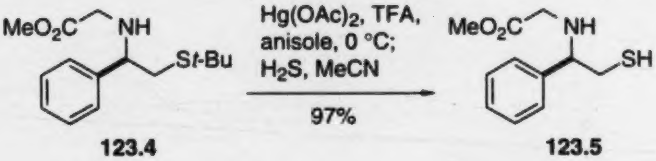
5 min to a stirred and cooled (ice-water bath) solution of **123.1** (1.0858 g, 5.19 mmol) and Et_3N (1.60 mL, 11.48 mmol) in dry PhH (12 mL) (N_2 atmosphere). The cooling bath was removed and the mixture was stirred for 1 h, by which time all of the starting material had reacted (TCL control, silica gel, EtOAc). The mixture was partitioned between EtOAc (50 mL) and H_2O (15 mL) and the organic phase was washed with H_2O (2 x 10 mL) and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in MeCN (5 mL) and cooled (ice-water bath), and $RuCl_3 \cdot 3H_2O$ (*ca.* 2 mg) and $NaIO_4$ (1.6849 g, 7.88 mmol) were added, followed by H_2O (7.5 mL). The cooling bath was removed and stirring was continued for 1.25 h, by which time all **123.2** had reacted (TCL control, silica gel, 50:50 EtOAc-hexanes). (Compound **123.2** is best visualized by u.v.) The mixture was partitioned between EtOAc (75 mL) and H_2O (50 mL), and the aqueous phase was extracted with EtOAc (2 x 25 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 40:60 EtOAc-hexanes, gave **123.3** (1.2248 g, 87%) as a pure (^{1}H NMR DMC XVII-111-A) white solid: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 3.65 and 3.97 (ABq, $\Delta\nu_{AB}$ = 116.5 Hz, J = 18.1 Hz, 2 H) overlapping a s at δ 3.71 (3 H), 4.41 (t, J = 8.6 Hz, 1 H), 4.84 (dd, J = 8.8, 7.0 Hz, 1 H), 5.24 (dd, J = 8.4, 7.0 Hz, 1 H), 7.37-7.47 (m, 5 H).

Methyl 2-[[*N*-[2-(*t*-butylsulfanyl)-1(*R*)-phenyl]ethyl]amino]acetate (123.4)



A solution of *t*-BuSH (0.18 mL, 1.60 mmol) in THF (2 mL) was added by cannula over *ca.* 1 min to a stirred suspension of NaH (0.0411 g, 1.84 mmol) in THF (1 mL), with additional THF (2 x 0.5 mL) used as a rinse (N_2 atmosphere). The mixture was stirred for 10 min and a solution of **123.3** (0.2205 g, 0.81 mmol) in THF (2 mL) was added by cannula, with additional THF (2 x 0.5 mL) used as a rinse. The mixture was stirred for 1 h and then aqueous H_2SO_4 (10%) (5 mL) was added, and stirring was continued for 1 h. The resulting mixture was neutralized with saturated aqueous $NaHCO_3$ and combined with EtOAc (50 mL). The organic phase was washed with saturated aqueous NaCl (15 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1.5 x 15 cm), using 30:70 EtOAc-hexanes, gave **123.4** (0.1121 g, 49%) as a pure (^{1}H NMR DMC XVI-119-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.33 (s, 9 H), 2.40-2.64 (broad s, 1 H), 2.76 (dd, *J* = 12.5, 9.4 Hz, 1 H), 2.86 (dd, *J* = 12.5, 4.4 Hz, 1 H), 3.20 and 3.33 (ABq, $\Delta\nu_{AB}$ = 50.4 Hz, *J* = 17.4 Hz, 2 H), 3.69 (s, 3 H), 3.78 (dd, *J* = 9.4, 4.4 Hz, 1 H), 7.24-7.38 (m, 5 H).

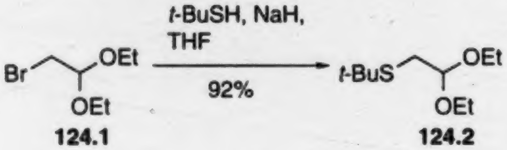
Methyl 2-[[*N*-[2-mercapto-1-(*R*)-phenyl]ethyl]amino]acetate (123.5)



A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **123.4** (0.8190 g, 0.29 mmol), followed by $Hg(OAc)_2$ (0.0927 g, 0.29 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (5 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad

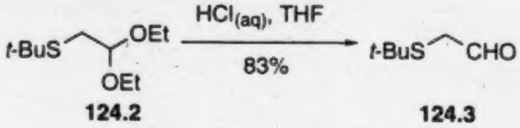
(1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated to give **123.5** (0.0634 g, 97%) as a pure (^1H NMR DMC XVI-113-A), colorless oil: Compound **123.5** is easily oxidized and should be used immediately. It had ^1H NMR ($CDCl_3$, 360 MHz) δ 1.80-2.40 (broad s, 2 H), 2.69 (dd, J = 13.3, 10.1 Hz, 1 H), 2.88 (dd, J = 13.3, 4.8 Hz, 1 H), 3.21 and 3.37 (ABq, $\Delta\nu_{AB}$ = 54.1 Hz, J = 18.3 Hz, 2 H), 3.70 (s, 3 H), 3.76 (dd, J = 10.1, 4.8 Hz, 1 H), 7.26-7.38 (m, 5 H).

2-(*t*-Butylthio)acetaldehyde diethyl acetal (124.2)



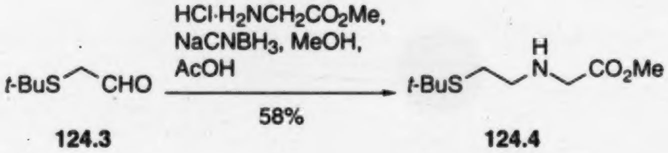
t-BuSH (15.0 mL, 133.05 mmol) was added by syringe pump over *ca.* 20 min to a stirred and cooled (ice-water bath) suspension of NaH (3.3601 g, 140.00 mmol) in THF (100 mL) (N_2 atmosphere). The mixture was stirred for 30 min and then **124.1** (20 mL, 132.94 mmol) was added by syringe pump over *ca.* 20 min. The resulting mixture was stirred and refluxed for 3 h, cooled to room temperature, and partitioned between saturated aqueous NaCl (50 mL), H_2O (25 mL), and EtOAc (50 mL). The aqueous phase was extracted with EtOAc (2 x 40 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated. The residual liquid was distilled under reduced pressure (0.07 mm Hg) to give **124.2** (25.2379 g, 92%) as a pure (^1H NMR DMC XVI-165-A), colorless liquid: bp 53-55 °C; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.20 (t, J = 7.0 Hz, 6 H), 1.30 (s, 9 H), 2.73 (d, J = 5.7 Hz, 2 H), 3.49-3.71 (m, 4 H), 4.59 (t, J = 5.73 Hz, 1 H).

(*t*-Butylthio)acetaldehyde (124.3)



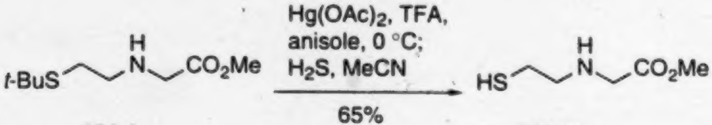
Aqueous HCl (10%) (2 mL) was added to a stirred solution of **124.2** (1.3146, 6.37 mmol) in THF (20 mL). Stirring was continued for 12 h, and then EtOAc (40 mL) was added. The organic phase was washed with saturated aqueous NaCl (10 mL), dried (MgSO4), and evaporated to give **124.3** (0.6971 g, 83%) as a pure (^{1}H NMR DMC XVI-175-A), colorless liquid: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.32 (s, 9 H), 3.26 (d, *J* = 2.0 Hz, 2 H), 9.55 (t, *J* = 2.0 Hz, 1 H).

N-[(2-*t*-butylsulfanyl)ethyl]glycine methyl ester (124.4)



$NaCNBH_3$ (0.3201 g, 5.09 mmol) was added to a stirred solution of **124.3** (0.6716 g, 5.08 mmol) and HClGlyOMe (0.6375 g, 5.08 mmol) in MeOH (25 mL) and AcOH (0.25 mL). The mixture was stirred for 3 h, evaporated, and partitioned between saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL) and the combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 50:50 EtOAc-hexanes, gave **124.4** (0.6053 g, 58%) as a pure (^{1}H NMR DMC XVI-169-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.30 (s, 9 H), 1.89 (s, 1 H), 2.67 (t, *J* = 6.1 Hz, 2 H), 2.80 (t, *J* = 6.1 Hz, 2 H), 3.42 (s, 2 H), 3.71 (s, 3 H).

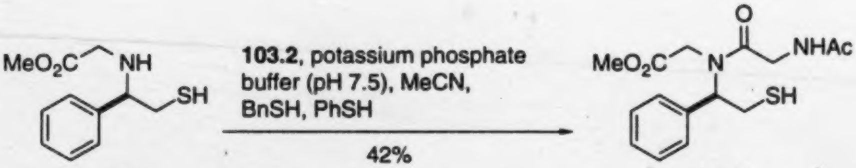
N-(2-Mercaptoethyl)glycine methyl ester (124.5)



124.4 **124.5**

A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **124.4** (0.2730 g, 1.33 mmol), followed by $Hg(OAc)_2$ (0.4237 g, 1.33 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (5 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad (1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated to give **124.5** (0.1298 g, 65%) as a pure (^{1}H NMR DMC XVI-171-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.80-2.40 (broad s, 2 H), 2.67 (t, *J* = 6.0 Hz, 2 H), 2.84 (t, *J* = 6.0 Hz, 2 H), 3.26 (s, 2 H), 3.74 (s, 3 H).

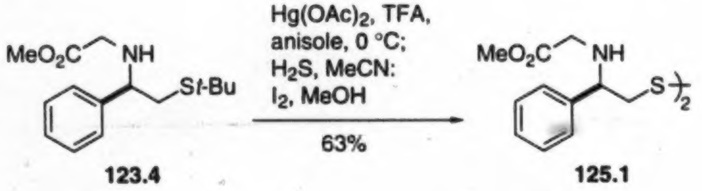
N-Acetylglycyl-*N*-[2-mercapto-1-(*R*)-phenylethyl]glycine methyl ester 123.6



123.5 **123.6**

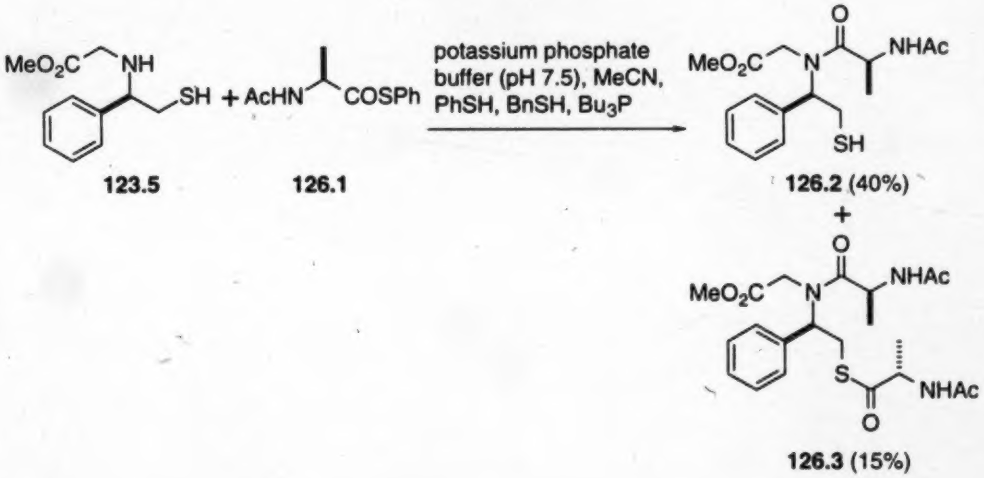
Sodium phosphate buffer[110] (1 mL, pH 7.5), BnSH (*ca.* 0.05 mL), and PhSH (*ca.* 0.05 mL) were added to a stirred solution of **123.5** (0.0419 g, 0.12 mmol) and **103.2** (0.0276 g, 0.12 mmol) in MeCN (2 mL) (N_2 atmosphere). Stirring was continued for 19 h, and the mixture was partitioned between EtOAc (50 mL), and H_2O (5 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1 x 20 cm), using EtOAc, gave **123.6** (0.0152 g, 42%) as a pure (^1H NMR DMC XVII-37-A), colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.56-2.43 [m, 4 H, including a t at δ 1.59 (J = 8.4 Hz), a s at δ 2.05, a s at δ 2.06, and a t at δ 2.39 (J = 8.5 Hz)], 2.96-3.14 (m, 2 H), 3.46-4.70 [m, 7 H, including an ABq at δ 3.50 and 3.90 ($\Delta\nu_{AB}$ = 160.7 Hz, J = 17.1 Hz) overlapping a s at δ 3.56, a s at δ 3.67, and a s at δ 3.80, a dABq at δ 4.00 and 4.05 ($\Delta\nu_{AB}$ = 17.52 Hz, J = 17.4, 4.0 Hz), and a dABq at δ 4.51 and 4.65 ($\Delta\nu_{AB}$ = 55.9 Hz, J = 17.5, 3.6 Hz)], 5.06-5.95 [m, 1 H, including a t at δ 5.09 (J = 7.2 Hz), and a t at δ 5.91 (J = 7.7 Hz)], 6.65 (s, 1 H), 7.20-7.42 (m, 5 H); exact mass (electrospray) *m/z* calcd for $C_{15}H_{20}N_2NaO_4S$ (M + Na) 347.1041, found 347.1049.

Methyl 2-[[*N*-[2-mercapto-1-(*R*)-phenyl]ethyl]amino]acetate disulfide (125.1)



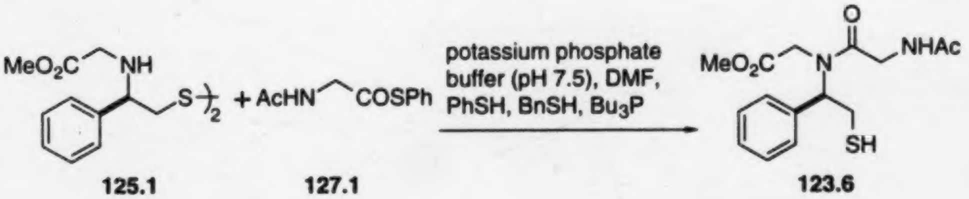
A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **123.4** (0.3448 g, 1.33 mmol), followed by $Hg(OAc)_2$ (0.3905 g, 1.33 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (5 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad (1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated to give a residue. The residue was dissolved in MeOH (10 mL) and titrated with saturated methanolic I_2. The resulting mixture was stirred for 10 min and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using EtOAc, gave **125.1** (0.1731 g, 63%) as a pure (^1H NMR DMC XVII-135-A), colorless oil: ^1H NMR ($CDCl_3$, 360 MHz) δ 2.35-2.65 (broad s, 1 H), 2.90 (dd, J = 13.5, 8.9 Hz, 1 H), 3.00 (dd, J = 13.5, 4.4 Hz, 1 H), 3.22 and 3.34 (ABq, $\Delta\nu_{AB}$ = 45.9 Hz, J = 17.4 Hz, 2 H), 3.69 (s, 3 H), 4.10 (dd, J = 8.9, 4.4 Hz, 1 H), 7.24-7.37 (m, 5 H).

***N*-Acetylalanyl-*N*-[2-mercapto-1-(*R*)-phenylethyl]glycine methyl ester (126.2) and *N*-Acetylalanyl-*N*[-2-mercapto-1-(*R*)-phenylethyl]glycine-S-[*N*-acetylalanyl]methyl ester (126.3)**



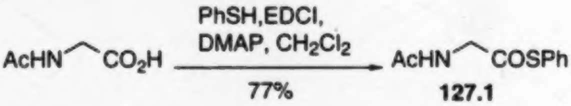
Sodium phosphate buffer[110] (0.75 mL, pH 7.5), BnSH (*ca.* 0.05 mL), and PhSH (*ca.* 0.05 mL) were added to a stirred solution of **123.5** (0.0419 g, 0.12 mmol) and **126.1** (0.0276 g, 0.12 mmol) in MeCN (1.5 mL) (N_2 atmosphere). Stirring was continued for 20 h, and the mixture was evaporated. Flash chromatography of the residue over silica gel (1 x 15 cm), using 90:10 EtOAc-MeOH, gave **126.2** (0.0264 g, 40%) as a pure (^{1}H NMR DMC XVII-79-A), colorless oil and **126.3** (0.0149 g, 15%) as a pure (^{1}H NMR DMC XVII-79-B), colorless oil: Compound **126.2** had: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.30-2.23 [m, 7 H, including a d at δ 1.36 (*J* = 8.2 Hz), a d at δ 1.48 (*J* = 8.2 Hz), a s at δ 1.98, a s at δ 2.03, and a t at δ 2.18 (*J* = 4.0 Hz)], 2.96-3.37 (m, 2 H), 3.42-5.48 (m, 7 H, including a s at δ 3.58 and a s at δ 3.68), 5.80-6.58 [m, 1 H, including a t at δ 5.85 (*J* = 6.0 Hz), and a t at δ 6.49 (*J* = 6.5 Hz)], 7.20-7.40 (m, 5 H); exact mass (electrospray) *m/z* calcd for $C_{16}H_{22}N_2NaO_4S$ (M + Na) 361.1198, found 361.1205. Compound **126.3** had: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.84-2.08 (m, 12 H), 3.38-5.42 (m, 10 H, including a s at δ 3.46 and a s at δ 3.63), 5.83-6.49 (m, 2 H), 7.20-7.44 (m, 5 H); exact mass (electrospray) *m/z* calcd for $C_{21}H_{29}N_3NaO_6S$ (M + Na) 474.1675, found 474.1670.

Typical procedure used for the coupling reactions described in Tables 16 and 17; illustrated by reference to reaction between 125.1 and 127.1 (entry 4, Table 16).



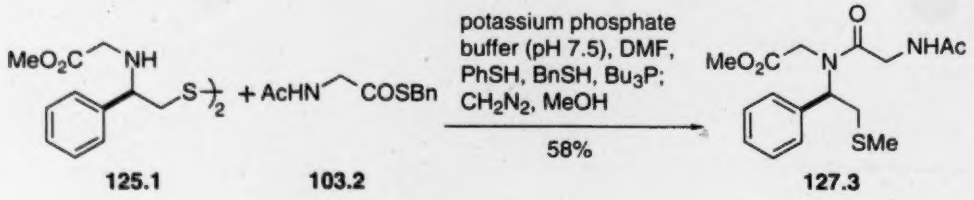
Bu_3P (80 μL, 0.32 mmol) was injected into a suspension of **125.1** (0.0236 g, 0.053 mmol) in DMF (0.4 mL) and sodium phosphate buffer (0.4 mL, pH 7.5), which caused a solution to form within 30 sec (N_2 atmosphere). The mixture was stirred for 5 min and **127.1** (0.0167 g, 0.080 mmol) was added, followed by BnSH (28 µL, 0.24 mmol), and PhSH (24 µL, 0.24 mmol). The reaction was monitored by HPLC by injecting 2 µL of the mixture onto a Hewlett Packard reversed phase C-8 column (4.6 x 200 mm) and eluting the compounds using a linear gradient changing from 20% solvent B to 55% solvent B over 25 min (flow rate = 2 mL/min, λ = 214 nm). Solvent A = 100:0.1 H_2O-TFA, solvent B = 90:10:0.1 MeCN-H_2O-TFA. Under these conditions the following retention times were observed: **103.2**, 10.3 min (sharp peak); **127.1**, 7.3 min (sharp peak); **123.6**, 8.6 min (sharp peak); **123.5**, 7.5 min (broad peak); BnSH, 17.2 min (sharp peak); PhSH, 15.6 min (sharp peak).

N-Acetylglycine Thiophenyl ester (127.1)



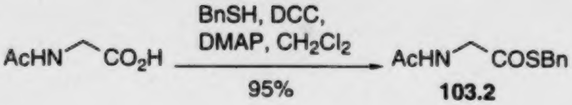
EDCI (1.6270 g, 8.49 mmol) and DMAP (*ca.* 20 mg) were added to a stirred suspension of AcGly (0.9938 g, 8.49 mmol) and PhSH (0.87 mL, 8.49 mmol) in dry CH_2Cl_2 (20 mL) (N_2 atmosphere). The mixture was stirred for 3 h and washed with 10% $HCl_{(aq)}$ (10 mL), saturated aqueous $NaHCO_3$ (10 mL), H_2O (10 mL) and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 80:20 EtOAc-hexanes, gave **127.1** (1.3692 g, 77%) as a pure (^1H NMR DMC XVI-37-A) white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3 H), 4.26 (d, *J* = 5.1 Hz, 2 H), 6.42 (s, 1 H), 7.38-7.42 (m, 5 H).

N-Acetylglycyl-*N*[-2-methylthio-1-(*R*)-phenylethyl]glycine methyl ester (127.3)


MeO2C
NH
S
2
+ AcHN
COSBn
potassium phosphate buffer (pH 7.5), DMF, PhSH, BnSH, Bu3P; CH2N2, MeOH
58%
O
MeO2C
N
NHAc
SMe
125.1
103.2
127.3

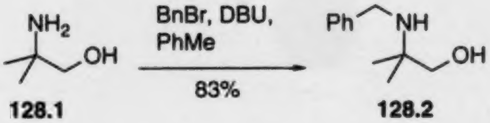
Bu_3P (130 µL, 0.52 mmol) was injected into a suspension of **125.1** (0.0291 g, 0.065 mmol) in DMF (4.33 mL) and sodium phosphate buffer (8.67 mL, pH 7.5), which caused a solution to form within 1 min (N_2 atmosphere). The mixture was stirred for 5 min and **103.2** (0.0217 g, 0.097 mmol) was added, followed by BnSH (46 µL, 0.39 mmol), and PhSH (40 µL, 0.39 mmol). The mixture was stirred for 14 h and was then acidified (pH *ca.* 3) with 6 M HCl. The resulting mixture was extracted with CH_2Cl_2 (5 x 20 mL), dried ($MgSO_4$) and evaporated. The residue was dissolved in MeOH (10 mL) and the stirred solution was titrated with ethanolic CH_2N_2. The mixture was stirred for 15 min, AcOH (1 drop) was added, and the mixture was evaporated. Flash chromatography of the residue over silica gel (1 x 20 cm), using 98:2 EtOAc-MeOH, gave **127.3** (0.0129 g, 58%) as a pure (^{1}H NMR DMC XVII-155-A), colorless foam: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.02-2.22 (m, 6 H, including a s at δ 2.05, a s at δ 2.06, a s at δ 2.17, and a s at δ 2.19), 2.95-3.16 (m, 2 H), 3.48-3.62 (m, 3 H, including a s at δ 3.51, and a s at δ 3.60), 3.73-4.50 [m, 4 H, including a d at δ 4.04 (*J* = 4.0 Hz), and a d at δ 4.47 (*J* = 3.6 Hz)], 5.06-6.08 [m, 1 H, including a t at δ 5.08 (*J* = 8.0 Hz), and a t at δ 6.03 (*J* = 7.8 Hz)], 7.22-7.43 (m, 5 H).

N-Acetylglycine Thiobenzyl ester (103.2)



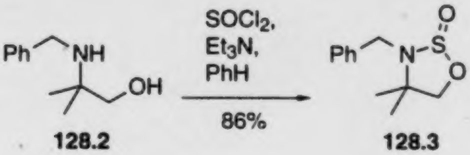
DCC (4.2131 g, 20.42 mmol) and DMAP (0.2271 g, 1.86 mmol) were added to a stirred suspension of AcGly (2.1733 g, 18.56 mmol) and BnSH (4.36 mL, 37.14 mmol) in dry CH_2Cl_2 (40 mL) (N_2 atmosphere). The mixture was stirred for 9 h and filtered through a grade D sintered glass frit. The filtrate was washed with H_2O (30 mL) and saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (4.5 x 20 cm), using 80:20 EtOAc-hexanes, gave **103.2** (3.9572 g, 95%) as a pure (^{1}H NMR DMC XIII-45-A) white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (s, 3 H), 4.13 (s, 2 H), 4.14 (d, *J* = 6.0 Hz, 2 H), 6.31 (s, 1 H), 7.22-7.32 (m, 5 H).

2-Benzylamino-2-methylpropan-1-ol (128.7)



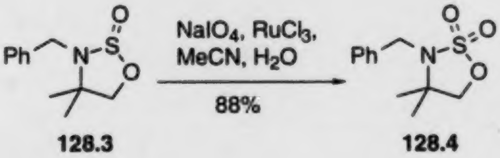
BnBr (12.46 mL, 104.76 mmol) was added by syringe pump over *ca.* 30 min to a stirred suspension of **128.1** (10 mL, 104.78 mmol) and DBU (15.70 mL, 104.98 mmol) in dry PhMe (100 mL) (N_2 atmosphere). The mixture was stirred for 1 h following addition of BnBr and then H_2O (100 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic extracts were dried ($MgSO_4$) and evaporated. The resulting liquid was distilled under reduced pressure (bp 119-121 °C, 0.001 mm Hg) to give **128.2** (15.5911 g, 83%) which solidified as a pure (^{1}H NMR DMC XVI-85-A) solid: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.18 (s, 6 H), 1.80-2.30 (broad s, 2 H), 3.37 (s, 2 H), 3.69 (s, 2 H), 7.26-7.38 (m, 5 H).

3-Benzyl-4,4-dimethyl-1,2,3-oxathiazolidine-2-oxide (128.3)



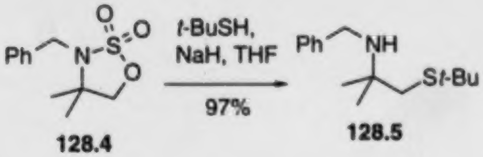
A solution of $SOCl_2$ (1.85 mL, 25.36 mmol) was added over *ca.* 20 min to a stirred and cooled (ice-water bath) solution of **128.2** (4.2849 g, 23.90 mmol) and Et_3N (7.35 mL, 52.73 mmol) in dry PhH (20 mL) (N_2 atmosphere). The cooling bath was removed and the mixture was stirred for 1 h and was then partitioned between EtOAc (50 mL) and H_2O (20 mL). The organic phase was washed with H_2O (20 mL) and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (3.5 x 25 cm), using 50:50 EtOAc-hexanes, gave **128.3** (4.6316 g, 86%) as a pure (^1H NMR DMC XVI-87-A) white solid: ^1H NMR ($CDCl_3$, 360 MHz) δ 1.24 (s, 3 H), 1.46 (s, 3 H), 4.12-4.66 [m, 4 H, including an ABq at δ 4.16 and 4.27 ($\Delta\nu_{AB}$ = 37.3 Hz, J = 14.6 Hz) overlapping an ABq at δ 4.20 and 4.64 ($\Delta\nu_{AB}$ = 158.3 Hz, J = 8.2 Hz)], 7.25-7.44 (m, 5 H).

3-Benzyl-4,4-dimethyl-1,2,3-oxathiazolidine-2,2-dioxide (128.4



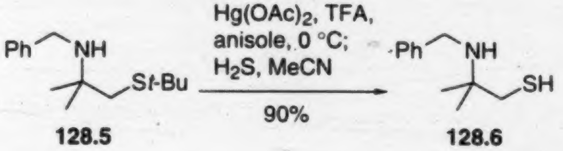
$RuCl_3{\cdot}3H_2O$ (*ca.* 5 mg) and $NaIO_4$ (1.3584 g, 6.35 mmol) were added to a stirred and cooled (ice-water bath) solution of **128.3** in MeCN (10 mL), followed by H_2O (15 mL). The cooling bath was removed and stirring was continued for 1 h, by which time all of the starting material had reacted (TCL control, silica gel, 50:50 EtOAc-hexanes). The mixture was partitioned between EtOAc (75 mL) and H_2O (20 mL), and the organic phase was washed with H_2O (2 x 25 mL) and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 50:50 EtOAc-hexanes, gave **128.4** (0.9037g, 88%) as a pure (^{1}H NMR DMC XVI-91-A) white solid: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.30 (s, 6 H), 4.27 (s, 4 H, two overlapping signals), 7.27-7.46 (m, 5 H).

Benzyl [1,1-dimethyl-2-(*t*-butylsulfanyl)]ethylamine (128.5)



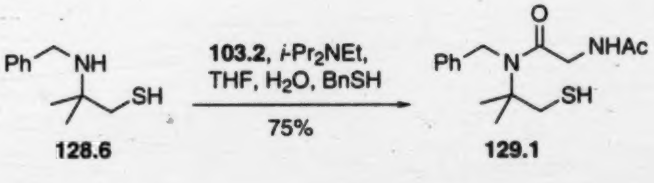
A solution of *t*-BuSH (0.13 mL, 1.15 mmol) in THF (2 mL) was added by cannula over *ca.* 1 min to a stirred suspension of NaH (0.0304 g, 1.27 mmol) in THF (1 mL), with additional THF (2 x 0.5 mL) used as a rinse (N_2 atmosphere). The mixture was stirred for 10 min and a solution of **128.4** (0.1391 g, 0.58 mmol) in THF (2 mL) was added by cannula, with additional THF (2 x 0.5 mL) used as a rinse. The mixture was stirred for 1 h and then aqueous H_2SO_4 (10%) (2 mL) was added, and stirring was continued for 1 h. The resulting mixture was neutralized with saturated aqueous $NaHCO_3$ and combined with EtOAc (50 mL). The organic phase was washed with saturated aqueous NaCl (15 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1.5 x 15 cm), using 80:20 EtOAc-hexanes, gave **128.5** (0.1420 g, 97%) as a pure (^{1}H NMR DMC XVI-95-B), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.24 (s, 6 H), 1.36 (s, 9 H), 1.60 (s, 1 H), 2.72 (s, 2 H), 3.71 (s, 2 H), 7.20-7.41 (m, 5 H).

2-Benzylamino-2-methylpropan-1-thiol (128.6)



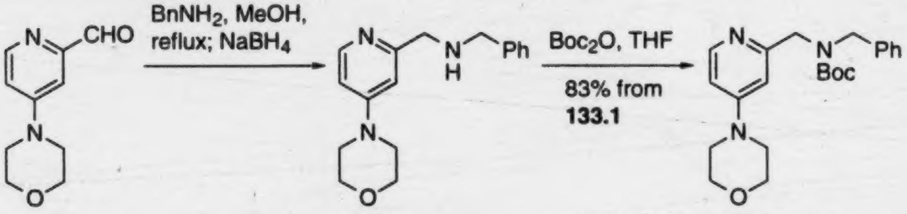
A cooled (ice-water bath) mixture of TFA (5 mL) and anisole (0.15 mL) was added to a cooled (ice-water bath) flask containing **128.5** (0.1239 g, 0.49 mmol), followed by $Hg(OAc)_2$ (0.1570 g, 0.49 mmol). The mixture was stirred for 15 min and then evaporated under reduced pressure (<0.1 mm Hg). The residue was dissolved in MeCN (5 mL) and H_2S was bubbled through the resulting stirred solution for 2 min. The black precipitate that formed was removed by filtration through a tightly packed (important) pad (1.5 x 3 cm) of Celite, topped with a 1 cm cotton plug, and the filtrate was evaporated under reduced pressure (<0.1 mm Hg). The residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (75 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated to give **128.6** (0.0867 g, 90%) as a pure (^1H NMR DMC XVI-97-A), colorless oil: ^1H NMR ($CDCl_3$, 360 MHz) δ 1.18 (s, 6 H), 1.61 (s, 2 H), 2.65 (s, 2 H), 3.63 (s, 2 H), 7.20-7.40 (m, 5 H).

2-[[*N*-(2-acetamido)acetyl]benzylamino]-2,2-dimethylethanethiol (129.1)



i-Pr_2NEt (0.15 mL, 0.86 mmol) was added to a stirred mixture of **128.6** (0.0867 g, 0.44 mmol) and **103.2** (0.1006 g, 0.45 mmol) in THF (3 mL) and H_2O (1 mL), followed by BnSH (*ca.* 0.05 mL) (N_2 atmosphere). The mixture was stirred for 39 h and combined with EtOAc (25 mL). The organic portion was removed, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1 x 15 cm), using 90:10 EtOAc-hexanes, gave **119.1** (0.0959 g, 75%) as a pure (^{1}H NMR DMC XV-99-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.23 (t, *J* = 9.0 Hz, 1 H), 1.42 (s, 6 H), 2.02 (s, 3 H), 3.18 (d, *J* = 9.0 Hz, 2 H), 4.09 (d, *J* = 4.1 Hz, 2 H), 4.65 (s, 2 H), 6.58 (s, 1 H), 7.12-7.38 (m, 5 H); exact mass (electrospray) *m/z* calcd for $C_{15}H_{22}N_2NaO_2S$ (M + Na) 317.1300, found 317.1305.

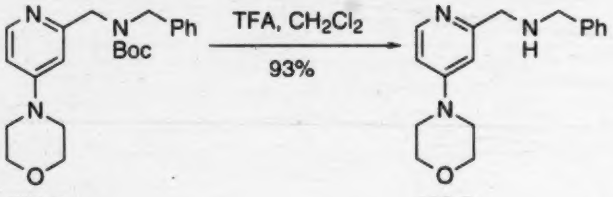
4-(4-Morpholino)-2-[(*N*-benzyl-*N*-*t*-butoxycarbonyl)aminomethyl]pyridine (133.3)



133.1 **133.2** **133.3**

$BnNH_2$ (0.34 mL, 3.11 mmol) was added to a stirred solution of **133.1** (0.5961 g, 3.10 mmol) in dry MeOH (25 mL), and the resulting mixture was stirred and refluxed for 1 h (N_2 atmosphere). The mixture was cooled (ice-water bath), and $NaBH_4$ (0.2347, 6.20 mmol) was added. The cold bath was removed and stirring was continued for 1 h. The mixture was evaporated and the residue was partitioned between H_2O (15 mL) and CH_2Cl_2 (30 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in THF (25 mL), Boc_2O (1.3523 g, 6.20 mmol) was added, and the mixture was stirred for 10 min and then evaporated. Flash chromatography of the residue over silica gel (2.5 x 25 cm), using 90:10 EtOAc-MeOH, gave **133.3** (0.9871 g, 83%) as a pure (^{1}H NMR DMC XVI-23-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.34-1.56 (m, 9 H, including a s at δ 1.40 and a s at δ 1.46), 3.24 (s, 4 H), 3.75-3.84 (m, 4 H), 4.30-4.58 (m, 4 H), 6.38-6.65 (m, 2 H), 7.15-7.35 (m, 5 H), 8.22 (d, *J* = 5.0 Hz, 1 H).

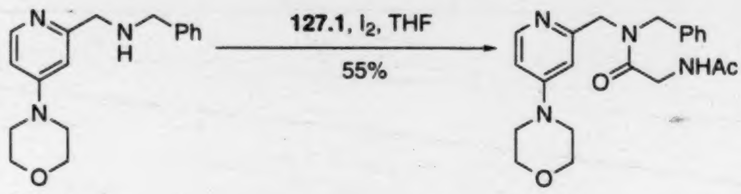
4-(4-Morpholino)-2-[(*N*-benzyl)aminomethyl]pyridine (133.2)



133.3 **133.2**

TFA (5.0 mL) was injected into a stirred solution of **133.3** (0.8166 g, 2.13 mmol) in CH_2Cl_2 (10 mL) (N_2 atmosphere). Stirring was continued for 1 h and the mixture was then evaporated to give a residue, which was partitioned between CH_2Cl_2 (40 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$) and evaporated to give **133.2** (0.5623 g, 93%) as a pure (^{1}H NMR DMC XVI-25-A), colorless oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.67 (s, 1 H), 3.21-3.58 (m, 4 H), 3.79-3.74 (m, 4 H), 3.80 (s, 4 H), 6.51 (dd, *J* = 5.9, 2.6 Hz, 1 H), 6.71 (d, *J* = 2.6 Hz, 1 H), 7.19-7.35 (m, 5 H), 8.22 (d, *J* = 5.9 Hz, 1 H).

N-Benzyl-*N*-[[4-(4-morpholino)pyrid-2-yl]methyl]-2-(acetamido)glycinamide (134.1)



133.2 **134.1**

I_2 (1.9543 g, 7.70 mmol) was added to a stirred solution of **133.2** (0.3117 g, 1.10 mmol) and **127.1** (0.2302 g, 1.10 mmol) in THF (10 mL). The mixture was stirred for 30 min, titrated with 1M $Na_2S_2O_{3(aq)}$, and partitioned between H_2O (10 mL) and CH_2Cl_2 (30 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in THF (10 mL) and Boc_2O (0.2401 g, 1.10 mmol) was added. The mixture was stirred for 10 min and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 90:10 EtOAc-MeOH, gave **134.1** (0.2281 g, 55%) as a pure (^{1}H NMR DMC XVI-53-A), colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96-2.03 (m, 3 H, including a s at δ 1.97 and a s at δ 2.00), 3.22-3.33 (m, 4 H), 3.72-3.79 (m, 4 H), 4.12-4.66 (m, 6 H, including a s at δ 4.14, a s at δ 4.18, a s at δ 4.52, a s at δ 4.58, a s at δ 4.61, and a s at δ 4.65), 6.58-6.69 (m, 1 H), 6.71-6.74 (m, 1 H), 7.19-7.37 (m, 5 H), 8.02-8.11 (m, 1 H); exact mass (electrospray) *m/z* calcd for $C_{21}H_{26}N_4NaO_3$ (M + Na) 405.1903, found 405.1902.

6 REFERENCES AND FOOTNOTES

1 Schnölzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pep. Protein Res.* **1992**, *40*, 180.

2 (a) Zoller, M. J.; Smith, M. *Methods Enzymol.* **1983**, *100*, 468. (b) Hutchison, C. A., III; Phillips, S.; Edgell, M. H.; Gillam, S.; Jahnke, P.; Smith, M. *J. Biol. Chem.* **1978**, *253*, 6551. (c) Knowles, J. R. *Science* **1987**, *236*, 1252.

3 (a) Cornish, W. V.; Mendel, D.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 621. (b) Bain, J. D.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R. *J.*

Am. Chem. Soc. **1989**, *111*, 8013.

4 Curtius, T. *J. Prakt. Chem.* **1881**, *24*, 239.

5 Fischer, E. *Chem. Ber.* **1902**, *35*, 1095.

6 Merrifield, R. B. *J. Amer. Chem. Soc.* **1963**, *85*, 2149.

7 (a) Bayer, E. *Angew. Chem. Int., Ed. Engl.* **1991**, *30*, 113. (b) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *48*, 11065.

8 Kent, S. B. H. *Ann. Rev. Biochem.* **1988**, *57*, 957.

9 See also, for example, reference 1.

10 *Peptide Chemistry at the Medical Research Council of Molecular Biology, Cambridge*; Compiled by Sheppard, R. C. Printed privately: Cambridge, 1997.

11 It is possible, although not routine, to go beyond 50 amino acid residues using solid phase peptide synthesis: See reference 1 and (a) Li, C. H.; Yamashiro, D. *J. Am. Chem. Soc.* **1970**, *92*, 7608. (b) Sharp, J. J.; Robinson, A. B.; Kamen, M. D. *J. Am. Chem. Soc.* **1973**, *95*, 6097. (c) Gutte, B.; Merrifield, R. B. *J. Am. Chem. Soc.* **1969**, *91*, 501.

12 See, for example: (a) Hirschmann, R.; Nutt, R. F.; Veber, D. F.; Vitali, R. A.; Varga, S. L.; Jacob, T. A.; Holly, F. W.; Denkewalter, R. G. *J. Am. Chem. Soc.* **1969**, *91*, 507, and earlier papers in the series. (b) Kaiser, E. T. *Acc. Chem. Res.* **1989**, *22*, 47. (c) Aimoto, S.; Mizoguchi, N.; Hojo, H.; Yoshimura, S. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 524. (d) Romovacek, H.; Dowd, S. R.; Kawasaki, K.; Nishi, N.; Hofmann, K. *J. Am. Chem. Soc.* **1979**, *101*, 6081. (e) Meienhofer, J. *Biopolymers* **1981**, *20*, 1761. (e) Yajima, H.; Fujii, N. *J. Am. Chem. Soc.* **1981**, *103*, 5867.

13 (a) Capon, B. *Q. Rev., Chem. Soc.* **1964**, *18*, 45. (b) Page, M.; Jencks, W. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 1678. (c) Kirby, A. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.

14 Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128.

15 Wieland, T.; Bokelmann, E.; Bauer, L.; Lang, H. U.; Lau, H. *Justus Liebigs Ann. Chem.* **1953**, *583*, 129.

16 (a) Brenner, M.; Zimmermann, J. P.; Wehrmüller, J.; Quitt, P.; Photaki, I. *Experientia* **1955**, *11*, 397. (b) Brenner, M.; Zimmermann, J. P.; Wehrmüller, J.; Quitt, P.; Hardtmann, A.; Schneider, W.; Beglinger, U. *Helv. Chim. Acta* **1957**, *40*, 1497.

17 Brenner, M. In *Peptides. Proceedings of the Eighth European Peptide Symposium*, Beyerman, H. C.; van den Linde, A.; van den Brink, W. M., Eds.; North-Holland: Amsterdam, 1967; pp 1.

18 Paulus, H. *Chem. Soc. Rev.* **1998**, *27*, 375.

19 (a) Proudfoot, A. E. I.; Rose, K.; Wallace, C. J. A. *J. Biol. Chem.* **1989**, *264*, 8764. (b) Wallace, C. J. A.; Corthésy, B. E. *Protein Eng.* **1986**, *1*, 23. (c) Homandberg, G. A.; Chaiken, I. M. *J. Biol. Chem.* **1980**, *255*, 4903. (d) Dyckes, D. F.; Creighton, T.; Sheppard, R. C. *Nature*, **1974**, *247*, 202. (e) *Cf.* Homandberg, G. A.; Mattis, J. A.; Laskowski, M., Jr. *Biochemistry* **1978**, *17*, 5220. (f) Offord, R. E. *Semisynthetic Proteins*; Wiley: New York, 1980.

20 (a) Mills, K. V.; Lew, B. M.; Jiang, S.-Q.; Paulus, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3543. (b) Lew, B. M.; Mills, K. V.; Paulus, H. *J. Biol. Chem.* **1998**, *273*, 15887.

21 Kemp, D. S. *Biopolymers* **1981**, *20*, 1793.

22 Fotouhi, N.; Galakatos, N. G.; Kemp, D. S. *J. Org. Chem.* **1989**, *54*, 2803.

23 Liu, C.-F.; Tam, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 4149.

24 Kemp, D. S.; Roberts, D.; Hoyng, C.; Grattan, J.; Vellaccio, F.; Reczek, J. In *Peptides: Chemistry, Structure, and Biology*, Walter, R., Ed.; Ann Arbor Science Publishers: Ann Arbor, Michigan, 1975; pp. 295.

25 Kemp, D. S.; Vellaccio, F., Jr. *J. Org. Chem.* **1975**, *40*, 3003.

26 A subsequent publication (see reference 21), indicated that products resulting from

O→*O* acyl transfer are also observed.

27 Kemp, D. S.; Gratten, J. A.; Reczek, J. *J. Org. Chem.* **1975**, *40*, 3465.

28 Imine formation from salicylaldehydes occurs with unusually large rate and equilibrium constants. See: Green, R. W.; Sleet, R. J. *Aust. J. Chem.* **1969**, *22*, 917, and references cited therein.

29 Although it is not clear from the published work (see reference 27), the acyl transfer presumably proceeds by initial *O*→*O* transfer between the phenolic hydroxyls, followed by *O*→*N* transfer.

30 Kemp, D. S.; Wang, S. W.; Busby, S., III; Hugel, G. *J. Am. Chem. Soc.* **1970**, *92*, 1043.

31 Kemp, D. S.; Vellaccio, F., Jr. *J. Org. Chem.* **1975**, *40*, 3464.

32 Kemp, D. S.; Kerkman, D. J.; Leung, S.-L.; Hanson, G. *J. Org. Chem.* **1981**, *46*, 490.

33 Worrall, D. E. *J. Am. Chem. Soc.* **1927**, *49*, 1598.

34 Kemp, D. S.; Choong, S.-L. H.; Pekaar, J. *J. Org. Chem.* **1974**, *39*, 3841.

35 Kemp, D. S.; Leung, S.-L.; Kerkman, D. J. *Tetrahedron Lett.* **1981**, *22*, 181.

36 (a) Kemp, D. S.; Kerkman, D. J. *Tetrahedron Lett.* **1981**, *22*, 185. (b) Kemp. D. S.; Buckler, D. R.; Galakatos, N. G.; Kirkman, D. *J. Org. Chem.* **1989**, *54*, 3853.

37 Simpson, R. B. *J. Am. Chem. Soc.* **1961**, *83*, 4711.

38 Kemp, D. S.; Galakatos, N. G. *J. Org. Chem.* **1986**, *51*, 1821.

39 Kemp, D. S.; Galakatos, N. G.; Bolin, D. In *Peptides: Synthesis, Structure, Function*, Rich, D.; Gross, E., Eds.; Pierce Chemical Company: Rockford, IL, 1981; pp 73.

40 Kemp, D. S.; Galakatos, N. G.; Bowen, B.; Tan, K. *J. Org. Chem.* **1986**, *51*, 1829.

41 March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*;

4th ed.; Wiley: New York, 1992; p 211.

42 Kemp, D. S.; Carey, R. I.; Dewan, J. C.; Galakatos, N. G.; Kerkman, D.; Leung, S.-L. *J. Org. Chem.* **1989**, *54*, 1589.

43 (a) Menger, F. M.; Smith, J. H. *J. Am. Chem. Soc.* **1972**, *94*, 3824. (b) Johnson, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237.

44 Although the data in Tables 4 and 5 suggest that DMSO would be a kinetically unsuitable solvent, it was, in fact, the only solvent that allowed acyl transfer at an acceptable rate, presumably because the reaction requires participation of a strong hydrogen-bonding acceptor (Kemp. D. S. In *Peptides. Proceedings of the 11th European peptide Symposium*, Nesvadba, H., Ed.; North Holland: Amsterdam, 1972; pp 1).

45 In earlier publications (see references 35 and 40), an EM of between 0.1 and 0.5 M was reported for this system. Presumably the more recent value of 3-14 M is a more accurate measurement.

46 Hanson, G.; Kemp, D. S. *J. Org. Chem.* **1981**, *46*, 5441.

47 Kemp, D. S.; Buckler, D. R. *Tetrahedron Lett.* **1991**, *32*, 3009.

48 Although it is not stated in the original publication, I assume that only extended conformations of the transition state are accessible with this template.

49 Kemp, D. S.; Buckler, D. R. *Tetrahedron Lett.* **1991**, *32*, 3013.

50 Kemp, D. S.; Galakatos, N. G.; Dranginis, S.; Ashton, C.; Fotouhi, N.; Curran, T. P. *J. Org. Chem.* **1986**, *51*, 3320.

51 *Cf.* (a) Khurgin, Yu. I.; Dmitrieva, M. G. *Tetrahedron* **1965**, *21*, 2305. (b) Pless, J.; Boissonnas, R. A. *Helv. Chim. Acta* **1963**, *48*, 1609.

52 McBride, B. J.; Kemp, D. S. *Tetrahedron Lett.* **1987**, *28*, 3435.

53 (a) Brois, S. J.; Pilot, J.; Barnum, H. *J. Am. Chem. Soc.* **1970**, *92*, 7629. (b) Hiskey, R. G.; Li, C., Vunnam, R. *J. Org. Chem.* **1975**, *40*, 950. (c) Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 1370.

54 Kemp, D. S.; Fotouhi, N. *Tetrahedron Lett.* **1987**, *28*, 4637.

55 Prepared (see reference 22) by reaction of **29.5** (R = OAc) with the cyclohexylamine salt of Cbz-L-Cys(Scm).

56 Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1989**, *54*, 3640.

57 In other applications (see reference 22) of the revised *Prior Thiol Capture Strategy* (see Scheme 36), non-cysteine side-chains, as well as the α-amino group of the template-linked *N*-terminal peptide segment (*cf.* **36.1**) were also protected (see reference 58).

58 Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1993**, *58*, 2216.

59 Kemp has explored (see reference 56) the use of a novel thiazolidine protecting group as a substitute for Acm. This thiazolidine is potentially advantageous in that it can be hydrolyzed selectively under conditions that are not detrimental to the other thiol protecting groups in the peptide, thus releasing a free thiol group for reaction with ScmCl.

60 The problem of converting the SAcm into the SScm moiety in the presence of the unprotected nucleophilic side-chains of lysine (see references 22 and 58) and histidine (see reference 61) has been studied. Histidine functionalization by ScmCl is suppressed by using formic acid-acetic acid-DMF for conversion of a terminal Cys(Acm) into Cys(Scm).

61 Kemp, D. S; Carey, R. I. *Tetrahedron Lett.* **1991**, *32*, 2845.

62 Chalfoun, D. J. Ph.D. Dissertation, Massachusetts Institute of Technology, Massachusetts, 1996.

63 Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776.

64 Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; Benjamin: New York, 1966; Vol. 1, pp 259.

65 Leu-enkephalin is: YGGFL. Voet, D.; Voet, J. G. *Biochemistry*; 2nd ed.; Wiley:

New York, 1995; p 1274.

66 Muir, T. W.; Dawson, P. E.; Kent, S. B. H. *Methods Enzymol.* **1997**, *289*, 266.

67 Dawson, P. E.; Churchill, M. J.; Ghadiri, M. R.; Kent, S. B. H. *J. Am. Chem. Soc.* **1997**, *119*, 4325.

68 Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10068.

69 The thioesters in this case were in the form shown in Table 8, where the thiol component is a portion of the linker used for solid phase synthesis of the peptide segment in question. Cleavage from the solid support gave the thioesters LYRAX-SR directly, thereby avoiding the additional step of thioacid derivatization.

70 Lu, W.; Qasim, M. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 8518.

71 Canne, L. E.; Walker, S. M.; Kent, S. B. H. *Tetrahedron Lett.* **1995**, *36*, 1217.

72 *Cf.* Canne, L. E.; Ferré-D'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *117*, 2998.

73 Kent has developed an efficient procedure for the preparation of this compound. See reference 71.

74 Several other peptides besides those discussed in this Review have been made (see references 66 and 75), but no details are given in the original publications.

75 Canne, L. E.; Bark, S. J.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 5891.

76 Cotton, G. J.; Ayers, B.; Xu, R.; Muir, T. W. *J. Am. Chem. Soc.* **1999**, *121*, 1100.

77 Canne, L. E.; Botti, P.; Simon, R. J.; Chen, Y.; Dennis, E. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1999**, *121*, 8720.

78 Canne, L. E.; Winston, R. L.; Kent, S. B. H. *Tetrahedron Lett.* **1997**, *38*, 3361.

79 Canne, L. E.; Ferré-D'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *117*, 2998.

80 The exact form of the *C*-terminus at this point is not clear from the original

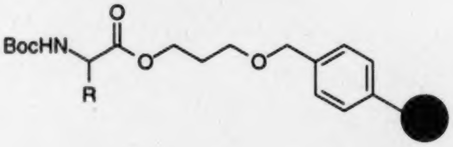
publication. The *C*-terminal peptide is either released from the resin as a thioester, in which case it is presumably used as such, or it is released in the form of a thioacid, in which case it is presumably derivatized as a thioester, and then elaborated.

81 The published results (see reference 75) do not indicate whether any attempt was made to separate the two peptides; hence, it is not clear if the *C*-terminal peptides used in the model ligations 4 and 5 (see Table 9) were pure stereoisomers.

82 Liu, C.-F.; Tam, J. P. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6584.

83 Tam, J. P.; Rao, C.; Liu, C.-F.; Shao, J. *Int. J. Pep. Protein Res.* **1995**, *45*, 209.

84 The following species has also been used as a solid support for these purposes. See reference 23.



85 Liu, C.-F.; Rao, C.; Tam, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 307.

86 For proline surrogates, see: Tam, J. P.; Miao, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9013 and references cited therein.

87 Tam, J. P.; Lu, Y.-A.; Liu, C.-F.; Shao, J. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12485.

88 Tam, J. P.; Yu, Q. *Biopolymers* **1998**, *46*, 319.

89 Heinrikson, R. L. *J. Biol. Chem.* **1971**, *246*, 4090.

90 Liu, C.-F.; Rao, C.; Tam, J. P. *Tetrahedron Lett.* **1996**, *37*, 933.

91 Zhang, L.; Tam, J. P. *Tetrahedron Lett.* **1997**, *38*, 3.

92 Bergman, M.; Zervas, L. *Biochem. Z.* **1928**, *203*, 280.

93 Rademann, J.; Grotli, M. M,; Bock, K. *J. Am. Chem. Soc.* **1999**, *121*, 5459, and

references therein.

94 Johnson, T.; Quibel, M.; Owen, D.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1993**, *4*, 369.

95 (a) Johnson, T.; Quibell, M.; Sheppard, R. C. *J. Pept. Sci.* **1995**, *1*, 11. (b) Offer, J.; Johnson, T.; Quibell, M. *Tetrahedron Lett.* **1997**, *38*, 9047.

96 Use of *n*-BuLi in place of *t*-BuLi gave poor results.

97 *Cf.* Kim, C. U.; Misco, P. F. *Tetrahedron Lett.* **1985**, *26*, 2027.

98 Gaunt, M. J.; Yu., J.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172.

99 The photolabile *p*-Methoxyphenylacyl ester was also considered for this purpose, but we did not examine it. *Cf.* Sevebryakov, E. P.; Suslova, L. M.; Kucherov, V. K. *Tetrahedron*, **1978**, *34*, 345.

100 Dawson, P. E.; Offer, J. *Org. Lett.* **2000**, *2*, 23.

101 Wagner, P. J.; Lindstrom, M. J. *J. Am. Chem. Soc.* **1987**, *109*, 3062.

102 Agami, C.; Couty, F.; Hamon, B.; Puchot, C. *Tetrahedron* **1990**, *46*, 7003.

103 *Cf.* Chauvel, E. N.; Coric, P.; Llorens-Cortès, C.; Wilk, S.; Roques, B. P.; Fournié-Zaluski, M.-C. *J. Med. Chem.* **1994**, *37*, 1339.

104 *Cf.* Weiss, B.; Stiller, R. L. *J. Org. Chem.* **1970**, *35*, 3543.

105 *Cf.* Deyrup, J. A.;, Moyer, C. L. *J. Org. Chem.* **1969**, *34*, 175.

106 *Cf.* Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.

107 Panek, J. S.; Masse, C. E. *J. Org. Chem.* **1998**, *63*, 2382.

108 Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2648.

109 Scriven, E. F. V. *Chem. Soc. Rev.* **1983**, *12*, 129.

110 Prepared by combining 16 mL of aqueous 0.2 M KH_2PO_4 and 84 mL of aqueous 0.2 M K_2HPO_4, and then diluting the mixture to 200 mL with H_2O.

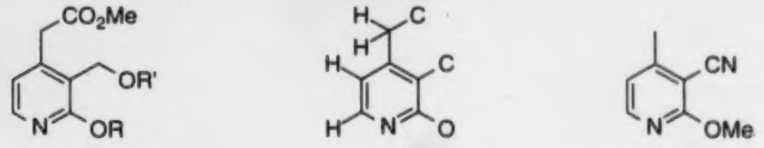
APPENDIX A

STUDIES ON THE PREPARATION OF 3,4-DISUBSTITUTED 2-METHOXYPYRIDINES

A.1 INTRODUCTION

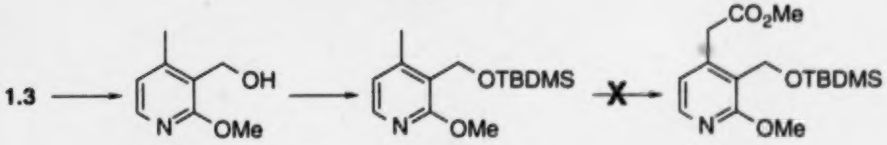
In connection with other work conducted in this laboratory, it was necessary to prepare compounds of type **1.1** (R = alkyl, R' = protecting group, see Scheme 1). The monocyclic substructure (see **1.2**) appears to be unknown,[1] and the preparation of an example of the ester **1.1** was initially troublesome. It was eventually decided that a route would be explored by way of 2-methoxy-4-methylpyridine-3-carbonitrile (**1.3**),[2] which we intended to deprotonate on the C(4) methyl, and convert into **2.3** (= **1.1**, R = Me, R' = TBDMS), along the lines shown in Scheme 2. The proposed route is based on the report[3] that **3.1** is convertible into **3.2** in high yield (see Scheme 3), under the conditions shown.

Scheme 1



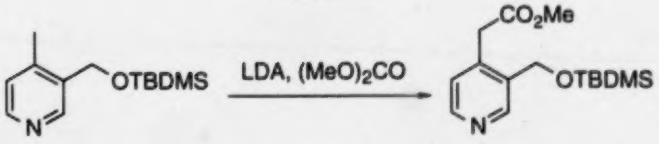
1.1 **1.2** **1.3**

Scheme 2



2.1 **2.2** **2.3**

Scheme 3

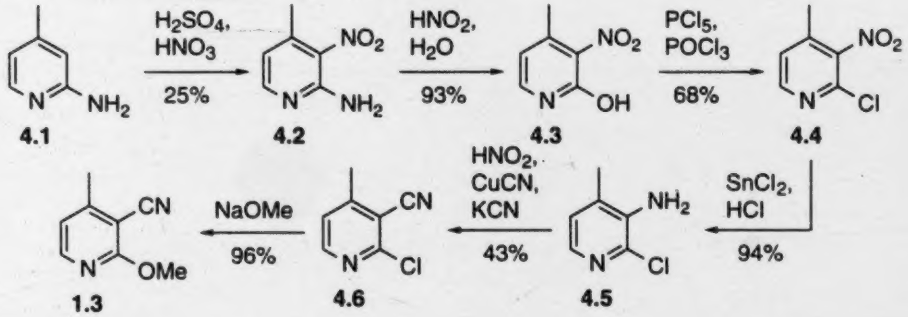


3.1 **3.2**

A.2 RESULTS AND DISCUSSION

Compound **1.3** had previously been prepared in our laboratory according to the procedure outlined in Scheme 4. Nitration[4] of commercially available and inexpensive **4.1** gave the 3-nitropyridine **4.2**, easily separated (25% yield) by steam distillation from the 5-nitro isomer that is also formed. Diazotization (93%) served to replace the amino group by hydroxyl (**4.2** → **4.3**)[5] and this, in turn, was replaced (68%) by chlorine (**4.3** → **4.4**).[5b] Reduction (**4.4** → **4.5**; 94%), using $SnCl_2$/hydrochloric acid,[6] and Sandmeyer reaction (HNO_2, CuCN, KCN, 30 °C) then gave **4.6** in 43% yield.[7] This route was easily done on a sufficiently large scale to afford 5-6 g batches of **4.6**. Finally, following a general procedure[8] for halide displacement, **4.6** was treated with NaOMe, leading to the formation of **1.3** in 96% yield.

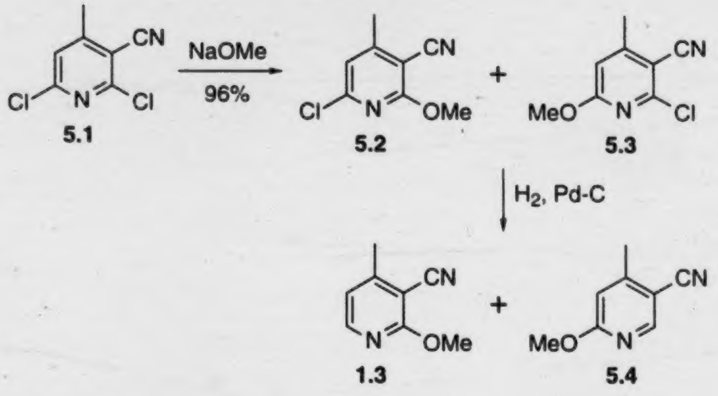
Scheme 4



For the present work, we employed an alternative, more concise route (Scheme 5) to nitrile **1.3**. In this approach, the readily accessible dichloro nitrile **5.1**[9] was treated with NaOMe (1 equiv.) to afford (92%) a 9:11 (^{1}H NMR) mixture of **5.2** and **5.3**, respectively. Hydrogenolysis then gave the easily separable chlorine-free compounds **5.4** and (in 36% yield) **1.3**.

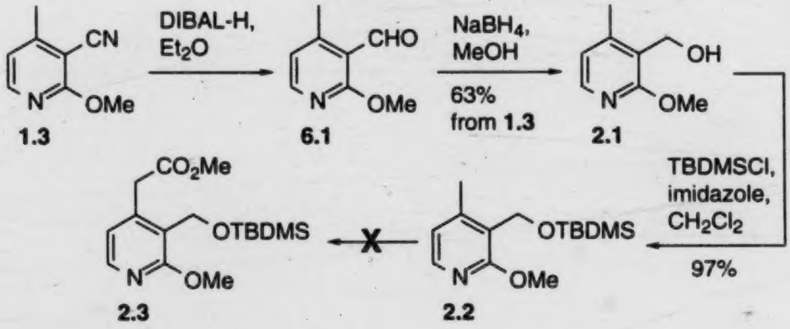
With nitrile **1.3** in hand, we began to investigate its conversion into **2.3** according to the plan outlined in Scheme 2. It was found that **1.3** could be easily reduced in 63%

Scheme 5



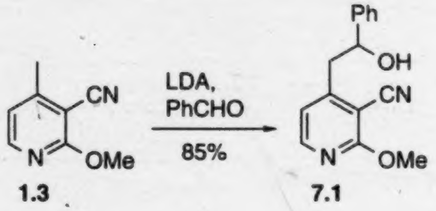
yield to alcohol **2.1** via the corresponding aldehyde intermediate (**6.1**, Scheme 6). The aldehyde was obtained by treatment of **1.3** with DIBAL-H, and was subsequently reduced to the desired alcohol using NaBH$_4$. The product was then silylated to afford **2.2** in 97% yield. Surprisingly, our attempts to acylate this compound (*cf.* Scheme 3) were uniformly unsuccessful, although we were easily able to repeat the conversion of **3.1** into **3.2**, exactly as reported[3] in the original literature. A control experiment, in which equimolar amounts of **2.2** and **3.1** were treated with LDA and (MeO)$_2$CO was also carried out. This experiment gave a complex mixture containing **3.2** and **2.2**, but no **2.3**, as judged by ^{1}H NMR analysis.

Scheme 6

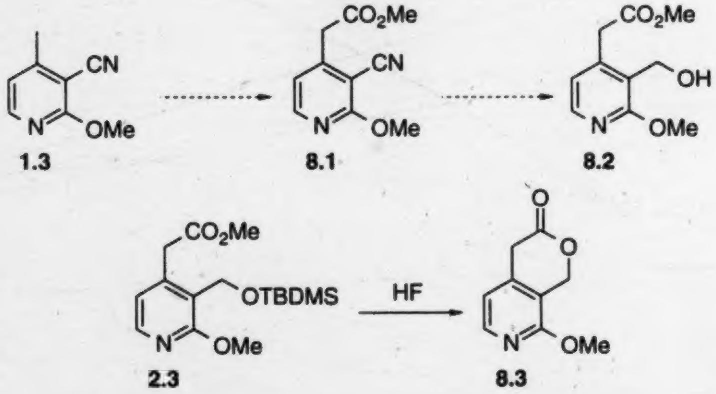


In contrast to the difficulties encountered in the attempted acylation of compound **2.2**, it had been found that nitrile **1.3** could be easily alkylated, by treatment with LDA and benzaldehyde, to afford **7.1** in 85% yield (Scheme 7). This observation led to the exploration of a less direct route to the desired target (**2.3**), which is outlined in Scheme 9 (see later). Direct acylation of **1.3** was not explored, as such a route would require reduction of the nitrile to a hydroxymethyl group *without* ring closure to form a lactone (**1.3** → **8.1** → **8.2**, Scheme 8). It was felt that this might be problematic and, in the event, treatment of **2.3** (obtained as described later) with HF resulted in spontaneous lactonization to give **8.3**.[10]

Scheme 7

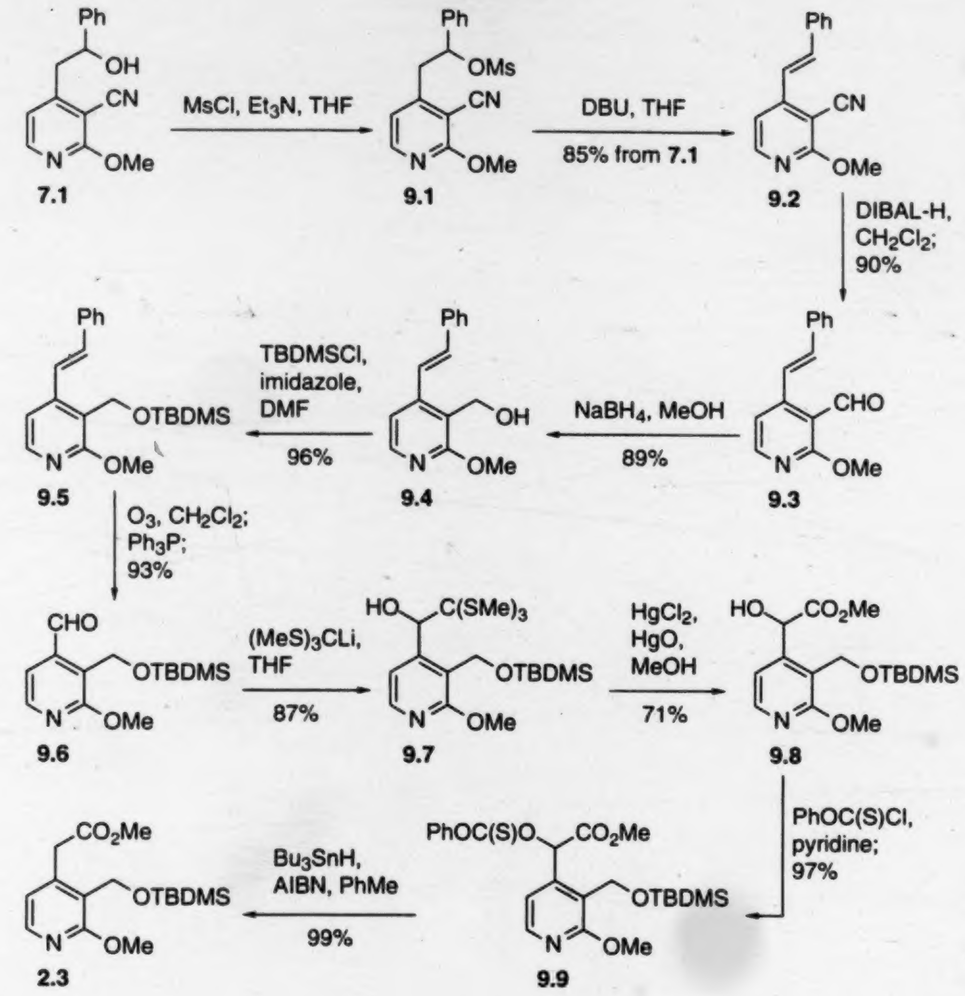


Scheme 8



Elaboration of **7.1** into **2.3** proceeded as outlined in Scheme 9. This Scheme represents a slightly modified version of a series of reactions that were first carried out by

Scheme 9



M. Pelesion in these laboratories. Mesylation (**7.1** → **9.1**, MsCl, Et_3N), followed by treatment with DBU, produced the *E*-olefin **9.2** (85% from **7.1**). Reduction to the aldehyde (**9.2** → **9.3**, DIBAL-H) was not straightforward, in the sense that, on a small scale (*ca.* 300 mg of **9.2**) the yield was 90%, but on an appreciably larger scale (*ca.* 5 g of **9.2**) reduction could not be driven to completion, even by use of a three-fold excess of the hydride, and recycling of recovered starting material was necessary in order to obtain a comparable yield. Further reduction with $NaBH_4$ then afforded the expected alcohol

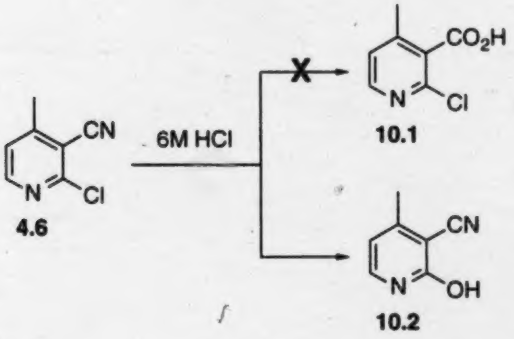
(**9.4**), and this was easily silylated (TBDMSCl, imidazole, 96%), giving the desired product **9.5**.

Ozonolytic cleavage of **9.5** produced aldehyde **9.6** (93%), in which the original C(4) carbon substituent of **1.3** is now electrophilic. Therefore, compounds **1.3** and **9.6** represent a complementary pair, with the C(4) carbon substituent of **1.3** able to behave as a nucleophile (after deprotonation) and, in **9.6**, as an electrophile.

Treatment of **9.6** with [tris(methylthio)]methyllithium[11] (**9.6** → **9.7**), and methanolysis[11] in the presence of $HgCl_2$ and HgO afforded ester **9.8**, from which the hydroxyl was removed by radical deoxygenation to give a suitably protected example of the compound type we required (**9.8** → **9.9**, 97%; **9.9** → **2.3**, 99%).

During the course of this work Pelison had tried to hydrolyze **4.6** to hydroxy acid **10.1** under the conditions (6M hydrochloric acid, reflux) described[7b] in the literature. The product has the reported mp (247 °C) but is, in fact, the hydroxy nitrile **10.2**.[12] Likewise, attempts to hydrolyze **1.3** and **9.2** under the same conditions (6M hydrochloric acid, reflux, 12 h) served only to replace the methoxy group by hydroxyl; in both cases the hindered nitrile group remained intact.

Scheme 10



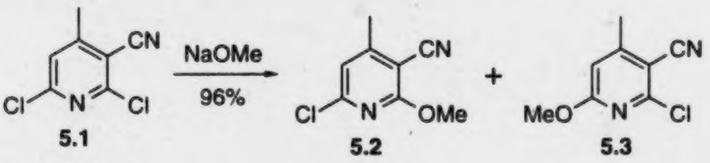
A.3 CONCLUSION

The synthesis of the required pyridine-based heterocylic compound **2.3** has been achieved. The relatively concise synthetic route to the desired compound that had been planned initially failed to provide the target due to the fact that intermediate **2.2** is not amenable to acylation. The route that was eventually employed to generate the compound, although longer than initially intended, gave yields at each step that were good to very good, and allowed the preparation of significant amounts of the target compound.

A.4 EXPERIMENTAL

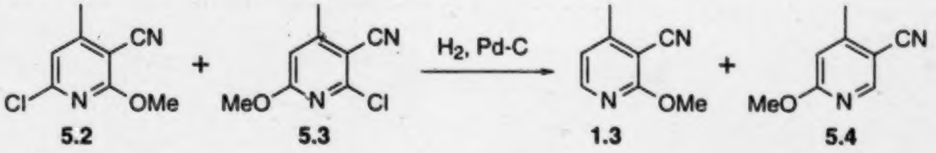
The same general procedures that were described in Chapter 1 apply.

2-Chloro-6-methoxy-4-methylpyridine-3-carbonitrile (5.3) and 6-Chloro-2-methoxy-4-methylpyridine-3-carbonitrile (5.2).



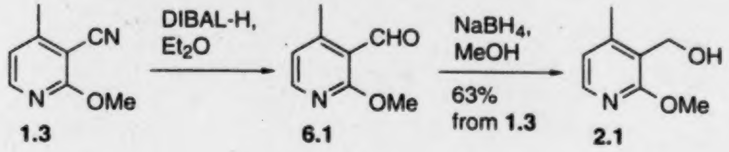
Na (4.5 g, 195.6 mmol) was added in small portions to a stirred solution of **5.1**[9] (36.6 g, 196 mmol) in MeOH (800 mL), a water bath being used to keep the temperature below 40 °C. After the last portion of Na had reacted the mixture was stirred for a further 30 min, and then most of the MeOH was evaporated. $CHCl_3$ (300 mL) and water (200 mL) were added to the resulting slurry, and the aqueous layer was extracted with $CHCl_3$ (2 x 100 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to give a cream-colored solid (32.8 g, 92%) that was a 9:11 mixture (^1H NMR) of **5.2** and **5.3**, ^1H NMR ($CDCL_3$, 200 MHz) δ 2.50 (two overlapping s, 3 H), 3.97 (s, 1.67 H), 4.05 (s, 1.37 H), 6.60 (s, 0.48 H), 6.90 (0.38 H). The material was filtered through a short column of flash chromatography silica gel, using 1;4 EtOAc-hexane, and used directly in the next step. The filtration is essential in order to remove a contaminant that poisons the catalyst in the next step.

6-Methoxy-4-methylpyridine-3-carbonitrile (5.4) and 2-Methoxy-4-methylpyridine-3-carbonitrile (1.3).



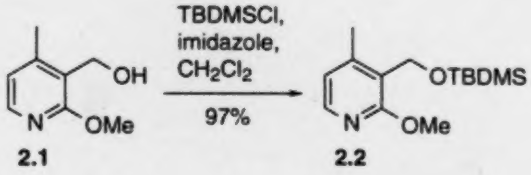
A suspension of the mixture of **5.2** and **5.3** (10.06 g, 55.09 mmol), NaOAc (4.498 g 54.84 mmol) and 10% Pd-C (0.9879 g) in MeOH (200 mL) was shaken with H_2 at 50 psi for 3 h (Parr shaker). The mixture was filtered, and most of the MeOH was evaporated. $CHCl_3$ (100 mL) and water (100 mL) were added to the residue, and the aqueous layer was extracted with $CHCl_3$ (2 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the resulting solid over silica gel (10 x 35 cm), using 1:4 EtOAc-hexane, gave **1.3** (2.8312 g, 35%) as a white solid, identical to material obtained from **4.6**, and gave **5.4** (3.3463g, 41%) also as a white solid. Compound **5.4** had: mp 95-98 °C; FTIR (CH_2Cl_2 cast) 1374, 2222 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.44 (d, *J* = 0.8 Hz, 3 H), 3.94 (s, 3 H), 6.65-6.68 (m, 1 H), 8.39 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.1 (q'), 54.4 (q'), 104.3 (s'), 111.9 (d'), 116.9 (s'), 152.3 (d'), 152.7 (s'), 166.6 (s'); exact mass *m/z* calcd for $C_8H_8N_2O$ 148.0636, found 148.0637. Anal. Calcd. for $C_8H_8N_2O$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.99; H, 5.39; N, 18.89.

3-Hydroxymethyl-2-methoxy-4-methylpyridine (2.1).



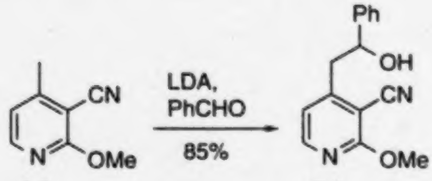
DIBAL-H (1 M in CH_2Cl_2, 9.98 mL, 9.98 mmol) was injected over *ca.* 10 min into a stirred and cooled (ice-water bath) solution of **1.3** (0.9238 g, 6.24 mmol) in Et_2O (20 mL) (Ar atmosphere). Stirring was continued for 1 h and then 4 M hydrochloric acid (5 mL) was added. The cold bath was removed and stirring was continued for 45 min. The aqueous phase was removed, and the organic phase was washed with water (5 mL) and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), and evaporated at atmospheric pressure (the product is volatile, and evaporation should not be done under reduced pressure). The residue was dissolved in MeOH (20 mL), and the solution was cooled (ice-water bath). $NaBH_4$ (0.4725 g, 12.49 mmol) was added, the cold bath was removed, and the mixture was stirred for 2 h, and then most of the MeOH was evaporated under reduced pressure. The residue was partitioned between EtOAc (50 mL) and water (25 mL), and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the resulting oil over silica gel (2.5 x 20 cm), using 4:1 EtOAc-hexanes, gave **2.1** (0.6010 g, 63%) as pure (^1H NMR), white crystals: mp 41-42 °C; FTIR (CH_2Cl_2 cast) 3600-3100 cm^{-1}; ^1H NMR (CD_2Cl_2, 200 MHz) δ 2.33 (s, 3 H), 3.36 (t, $J = 5.0$ Hz, 1 H), 3.88 (s, 3 H), 4.62 (d, $J = 5.0$ Hz, 2 H), 6.69 (d, $J = 5.2$ Hz, 1 H), 7.87 (d, $J = 5.2$ Hz, 1 H); ^{13}C NMR (CD_2Cl_2, 50.3 MHz) δ 18.4 (q'), 53.6 (q'), 56.3 (t'), 119.8 (d'), 121.6 (s'), 145.6 (d'), 148.6 (s'), 162.6 (s'); exact mass *m/z* calcd for $C_8H_{11}NO_2$ 153.0790, found 153.0791. Anal. Calcd. for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.47; H, 7.14; N, 9.18.

3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-2-methoxy-4-methylpyridine (2.2).



TBDMSCl (2.156 g, 14.31 mmol) and imidazole (0.9733 g, 14.30 mmol) were added to a stirred solution of **2.1** (0.8807 g, 7.15 mmol) in dry CH_2Cl_2 (30 mL) (Ar atmosphere). Stirring was continued for 45 min, by which time all of the starting material had reacted (TLC control; silica gel, 50:50 EtOAc-hexanes). The solution was washed with water (15 mL) and saturated aqueous NaCl (15 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the resulting oil over silica gel (2.5 x 20 cm), using 1:1 EtOAc-hexanes, gave **2.2** (1.6468 g, 97%) as a pure (^{1}H NMR), colorless oil: FTIR (CH_2Cl_2 cast) 1256 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 200 MHz) δ 0.07 (s, 6 H), 0.90 (s, 9 H), 2.37 (s, 3 H), 3.91 (s, 3 H), 4.57 (s, 2 H), 6.72 (d, *J* = 5.1 Hz, 1 H), 7.93 (d, *J* = 5.1 Hz, 1 H); ^{13}C NMR (CD_2Cl_2, 50.3 MHz) δ -5.3 (q'), 18.6 (q'), 18.6 (s'), 26.0 (q'), 53.5 (q'), 56.7 (t'), 119.8 (d'), 121.5 (s'), 145.8 (d'), 149.8 (s'), 162.3 (s'); exact mass *m/z* calcd for $C_{13}H_{22}NO_2Si$ (M - CH_3) 252.1420, found 252.1418. Anal. Calcd. for $C_{14}H_{25}NO_2Si$: C, 62.87; H, 9.42; N, 5.24. Found: C, 63.09; H, 9.49; N, 5.22.

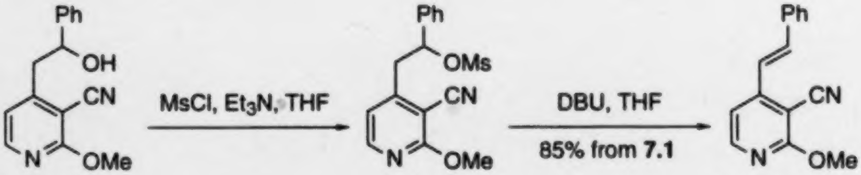
4-(2-Hydroxy-2-phenylethyl)-2-methoxypyridine-3-carbonitrile (7.1).



1.3 **7.1**

LDA was prepared by dropwise addition of BuLi (1.6 M solution in hexanes, 23 mL, 36.9 mmol) to a stirred and cooled (0 °C) solution of *i*-Pr_2NEt (5.2 mL, 36.9 mmol) in THF (100 mL). The solution was stirred for 15 min at 0 °C, cooled to -78 °C and then, after 10 min, a solution of **1.3** (2.75 g, 18.5 mmol) in THF (20 mL) was injected over *ca.* 20 min to produce a pale yellow solution. After 30 min at -78 °C, a solution of BnCHO (3.91 g, 36.9 mmol) in THF (10 mL) was injected, and stirring at -78 °C was continued for 15 min. The cold bath was removed, and stirring was continued for 2 h. Saturated aqueous NH_4Cl (30 mL) was added, and the mixture was extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (3.5 x 25 cm), using 2:3 EtOAc-hexane, gave **7.1** (4.0 g, 85%) as a colorless oil, ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.34 (br s, 1 H), 3.17 (d, J = 7.2 Hz, 2 H), 4.04 (s, 3 H), 5.02 (t, J = 7.2 Hz, 1 H), 6.85 (d, J = 4.8 Hz, 1 H), 7.28 - 7.40 (m, 5 H), 8.19 (d, J = 4.8 Hz, 1 H).

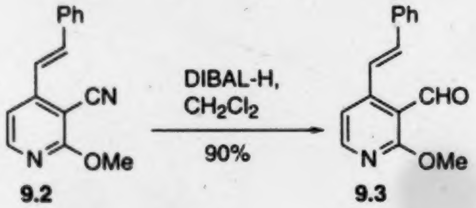
2-Methoxy-4-(2-phenylethenyl)pyridine-3-carbonitrile (9.2).



7.1 **9.1** **9.2**

Freshly distilled MsCl (2.8 mL, 36.16 mmol) was added dropwise to a stirred and cooled (0 °C) solution of **7.1** (6.11 g, 24.00 mmol) and Et_3N (6.6 mL, 47.35 mmol) in dry THF (200 mL). The cold bath was removed, and the mixture was stirred for a further 30 min, by which time a precipitate had formed. DBU (7.2 mL, 48.22 mmol) was injected, and stirring at room temperature was continued for 30 min. The mixture was partitioned between Et_2O and water, the organic layer was separated, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (5 x 20 cm), using 3:17 EtOAc-hexane, gave **9.2** (4.82 g, 85%) as a single (*E*) isomer: FTIR (CH_2Cl_2 cast) 1388, 1551, 1583, 2223 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.08 (s, 3 H), 7.25-7.62 [m, including d (1 H, *J* = 5.5 Hz) at δ 7.24, 8 H in all], 8.25 (d, *J* = 5.5 Hz, 1 H); ^{13}C NMR: δ 54.6 (q'), 94.9 (s'), 112.1 (d'), 114.5 (s'), 122.1 (d'), 127.7 (d'), 129.0 (d'), 129.6 (d'), 135.2 (s'), 137.5 (d'), 149.9 (d'), 151.1 (s'), 165.3 (s'); exact mass *m/z* calcd for $C_{15}H_{12}N_2O$ 236.0950, found 236.0946.

2-Methoxy-4-(2-phenylethenyl)pyridine-3-carbaldehyde (9.3).

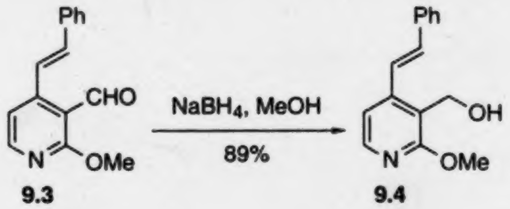


DIBAL-H (1 M in CH_2Cl_2, 2.0 mL, 2.0 mmol) was injected over *ca.* 5 min into a stirred and cooled (-78 °C) solution of **9.2** (324 mg, 1.37 mmol) in CH_2Cl_2 (10 mL). Stirring at -78 °C was continued for 1.5 h and then MeOH (0.5 mL), Celite (1 g), Na_2SO_4 (2 g), and a few drops of water were added. The cold bath was removed and the stirred mixture was allowed to warm to room temperature and filtered. Evaporation of the filtrate and flash chromatography of the residue over silica gel (2 x 15 cm), using 3:17 EtOAc-hexane, gave **9.3** (300 mg, 90%): FTIR (CH_2Cl_2 cast) 1678 cm^{-1}; ^1H NMR ($CDCl_3$, 300

MHz) δ 4.05 (s, 3 H), 7.22-7.43 [m, including d (1 H, J = 6.0 Hz) at δ 7.22, and d (1 H, J = 16.0 Hz) at δ 7.80, 5 H in all], 7.56-7.62 (m, 2 H), 8.19 (d, J = 16.0 Hz, 1 H), 8.25 (d, J = 6.0 Hz, 1 H), 10.56 (s, 1 H); ^{13}C NMR: δ 55.8 (q'), 116.3 (d'), 117.0 (s'), 126.4 (d'), 129.3 (d'), 130.7 (d'), 130.9 (d'), 137.9 (d'), 138.4 (s'), 150.5 (s'), 152.9 (d'), 193.4 (d'); exact mass *m/z* calcd for $C_{15}H_{13}NO_2$ 239.0946, found 239.0947.

When this reaction is done on a 5 g-scale only half of the material is reduced, even using an excess of DIBAL-H (3 equiv.) and/or a longer time (overnight). The total yield remains around 88%, after correction for recovered starting material.

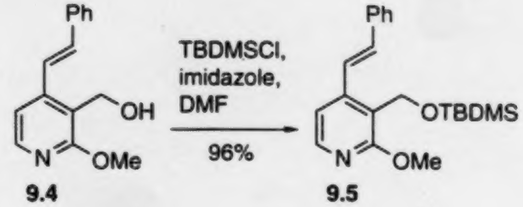
2-Methoxy-4-(2-phenylethenyl)pyridin-3-ylmethanol (9.4).



NaBH$_4$ (1.00 g, 26.0 mmol) was added in small portions to a stirred and cooled (0 °C) solution of **9.3** (1.97 g, 8.25 mmol) in MeOH (150 mL). The mixture was stirred for a further 1 h and then most of the MeOH was evaporated. Water (100 mL) and EtOAc (100 mL) were added, and the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (3 x 15 cm), using 1:3 EtOAc-hexane, gave **9.4** (1.77 g, 89%): FTIR (CH_2Cl_2 cast) 1495, 1633, 3355 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.25 (t, J = 6.8 Hz, 1 H), 4.02 (s, 3 H), 4.84 (d, J = 6.8 Hz, 2 H), 7.05-7.57 [m, including d (1 H, J = 6.0 Hz) at δ 7.08, 8 H in all], 8.05 (d, J = 6.0 Hz, 1 H); ^{13}C NMR: δ 53.9 (q'), 56.5 (t'), 114.3 (d'), 119.8 (s'), 123.3 (d'), 127.1 (d'), 128.8 (d'), 128.9 (d'), 135.0 (d'), 136.5 (s'), 145.7 (d'), 146.3 (s'), 162.9 (s'); exact mass *m/z* calcd for $C_{15}H_{15}N_2O$

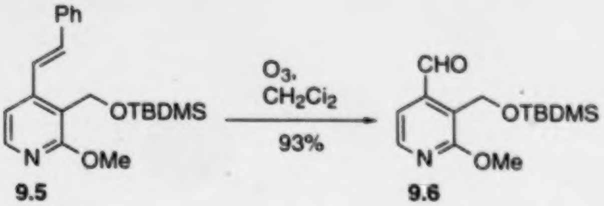
241.1103, found 241.1103.

3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-2-methoxy-4-(2-phenylethenyl)pyridine (9.5).



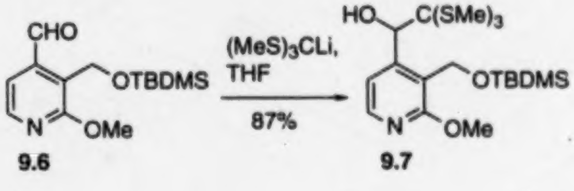
TBDMSCl (2.03 g, 13.5 mmol) in DMF (5 mL) was added to a stirred and cooled (0 °C) solution of **9.4** (3.26 g, 13.5 mmol) and imidazole (1.84 g, 27.0 mmol) in DMF (5 mL) at 0 °C. The ice bath was removed and the mixture was stirred at room temperature overnight. The mixture was diluted with Et_2O (40 mL), washed with water (2 x 10 mL) and saturated aqueous NaCl (10 mL), and dried (Na_2SO_4). Evaporation of the solvent gave pure (^{1}H NMR) **9.5** (4.6 g, 96%): FTIR (CH_2Cl_2 cast) 837, 1074, 1593 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 300 MHz) δ 0.12 (s, 6 H), 0.92 (s, 9 H), 3.97 (s, 3 H), 4.90 (s, 2 H), 7.18 (d, *J* = 6.0 Hz, 1 H), 7.20-7.44 (m, 4 H), 7.52-7.60 (m, 3 H), 8.08 (d, *J* = 6.0 Hz, 1 H); ^{13}C NMR (CD_2Cl_2) δ -5.1 (q'), 18.6 (s'), 26.1 (q'), 53.8 (q'), 56.6 (t'), 114.0 (d'), 120.2 (s'), 124.8 (d'), 127.4 (d'), 128.8 (d'), 129.2 (d'), 134.0 (d'), 137.3 (s'), 146.1 (d'), 147.3 (s'), 163.2 (s'); exact mass *m/z* calcd for $C_{20}H_{26}O_2Si$ (M - CH_3) 340.1733, found 340.1726.

3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-2-methoxypyridine-4-carbaldehyde (9.6).



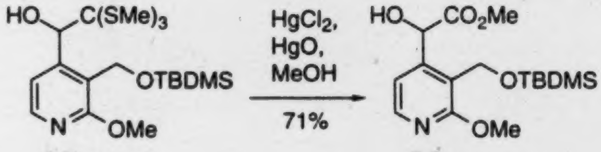
An ozone-oxygen stream was bubbled through a stirred and cooled (-78 °C) solution of **9.5** (52.3 mg, 0.147 mmol) in dry CH_2Cl_2 (3 mL) until a blue color appeared (about 10 min). Oxygen was passed through the solution for 5 min to remove the excess ozone, and Ph_3P (50 mg, 0.19 mmol) was added. The cold bath was removed, and the stirred mixture was allowed to warm to room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (1 x 15 cm), using 1:9 EtOAc-hexane, gave **9.6** (38.4 mg, 93%): FTIR (CH_2Cl_2 cast) 837, 1072, 1594, 1705 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 0.10 (s, 6 H), 0.88 (s, 9 H), 3.99 (s, 3 H), 5.05 (s, 2 H), 7.11 (d, *J* = 2.0 Hz, 1 H), 8.21 (d, *J* = 2.0 Hz, 1 H), 10.58 (s, 1 H); ^{13}C NMR (CD_2Cl_2) δ -4.8 (q'), 18.8 (s'), 26.4 (q'), 54.7 (q'), 56.8 (t'), 114.3 (d'), 125.2 (s'), 143.9 (s'), 147.2 (s'), 162.5 (s'), 192.7 (s'); exact mass *m/z* calcd for $C_{14}H_{23}NO_3Si$ 281.1447, found 281.1442.

1-[3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-2-methoxypyrid-4-yl]-2,2,2-[tris(methylthio)]ethanol (9.7).



BuLi (1.6 M in hexanes, 7.7 mL, 12.3 mmol) was added to a solution of tris(methylthio)methane (1.89 g, 12.3 mmol) in THF (80 mL) at -78 °C. The mixture was stirred for 30 min, and a solution of **9.6** (2.88 g, 10.25 mmol) in THF (45 mL plus 5 mL as a rinse) was added. The mixture was stirred for 15 min, the cold bath was removed and, after the mixture had warmed to room temperature, saturated aqueous NH_4Cl (20 mL), water (30 mL) and EtOAc (80 mL) were added. The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic extracts were washed with saturated aqueous NaCl, and dried (Na_2SO_4). Evaporation of the solvent and flash chromatography of the residue over silica gel (3.5 x 15 cm), using 2:3 EtOAc-hexane, gave **9.7** (3.88 g, 87%): FTIR (CH_2Cl_2 cast) 816, 1050, 1571, 1595, 3456 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 0.04 (s, 3 H), 0.12 (s, 3 H), 0.80 (s, 9 H), 2.10 (s, 9 H), 3.54 (s, 1 H), 3.92 (s, 3 H), 5.00 (AB q, J = 12.0, $\Delta\nu_{AB}$ = 8.3 Hz, 2 H), 5.56 (s, 1 H), 7.47 (d, J = 6.0 Hz, 1 H), 8.08 (d, J = 6.0 Hz, 1 H); ^{13}C NMR (CD_2Cl_2) δ -5.3 (q'), -4.9 (q'), 14.3 (q'), 18.5 (s'), 26.1 (q'), 53.8 (q'), 57.0 (t'), 72.9 (d'), 75.8 (s'), 118.1 (d'), 122.4 (s'), 145.4 (d'), 148.9 (s'), 162.0 (s'). A satisfactory mass spectrum could not be obtained.

Methyl 2-[3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-methyl]-2-methoxypyrid-4-yl]-2-hydroxyethanoate (9.8).

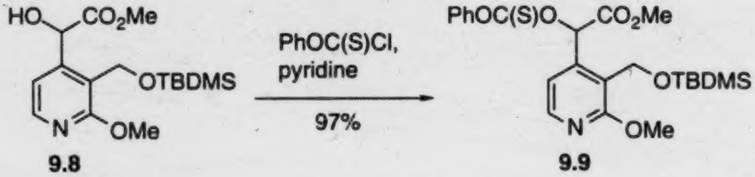


9.7 **9.8**

A mixture of **9.7** (3.15 g, 7.24 mmol), $HgCl_2$ (7.86 g, 28.96 mmol) and HgO (2.62g, 12.1 mmol) in 12:1 MeOH-water (170 mL) was stirred at room temperature for 4 h. The mixture was filtered and the solid residue was washed with CH_2Cl_2 (2 x 30 mL). The combined filtrates were diluted with water (150 mL) and extracted with CH_2Cl_2 (2 x 150 mL). The combined organic extracts were washed with saturated aqueous NaCl and dried ($MgSO_4$). Evaporation of the solvent and flash chromatography of the residue over

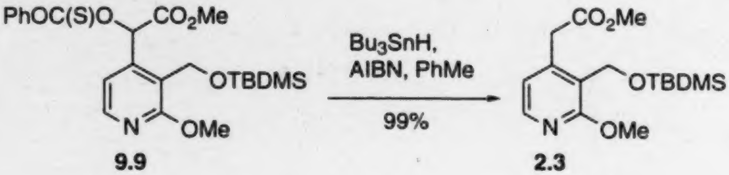
silica gel (3 x 15 cm), using 1:2 EtOAc-hexane, gave **9.8** (1.75 g, 71%): FTIR ($CHCl_3$ cast) 836, 1060, 1576, 1597, 1746, 3441 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.10 (s, 6 H), 0.89 (s, 9 H), 3.76 (s, 3 H), 3.94 (s, 3 H), 4.31 (d, J = 7.0 Hz, 1 H), 4.90 (AB q, J = 13.0, $\Delta\nu_{AB}$ = 9.0 Hz, 2 H), 5.57 (d, J = 7.0 Hz, 1 H), 6.85 (d, J = 5.0 Hz, 1 H), 8.10 (d, J = 5.0 Hz, 1 H); ^{13}C NMR: δ -5.4 (q'), -5.3 (q'), 18.3 (s'), 25.9 (q'), 52.9 (q'), 53.7 (q'), 56.3 (t'), 70.6 (d'), 116.2 (d'), 120.8 (s'), 146.6 (d'), 149.0 (s'), 161.9 (s'), 173.1 (s'); exact mass *m/z* calcd for $C_{12}H_{18}NO_5Si$ (M - *t*-Bu) 284.0954, found 284.0958.

Methyl 2-[3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-methyl]-2-methoxypyrid-4-yl]-2-[(phenoxy)thiocarbonyloxy]ethanoate (9.9).



Phenyl thiochloroformate (0.55 mL, 3.92 mmol) was added to a stirred solution of **9.8** (1.22 g, 3.565 mmol) and pyridine (1.45 mL, 17.8 mmol) in CH_2Cl_2 (50 mL) at room temperature. The mixture was stirred for 2 h, and the solvent was then evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:4 EtOAc-hexane, gave **9.9** (1.57 g, 97%): FTIR ($CHCl_3$ cast) 836, 1066, 1201, 1761 cm^{-1}; ^1H NMR (CD_2Cl_2, 200 MHz) δ 0.07 (s, 3 H), 0.11 (s, 3 H), 0.90 (s, 9 H), 3.75 (s, 3 H), 3.98 (s, 3 H), 4.95 (s, 2 H), 7.00-7.18 [m, including d (1 H, J = 6.0 Hz) at δ 7.04, 3 H in all], 7.28-7.50 (m, 3 H), 8.18 (d, J = 6.0 Hz, 1 H); ^{13}C NMR (CD_2Cl_2) δ -5.3 (q'), 18.6 (s'), 26.0 (q'), 53.2 (q'), 54.1 (q'), 56.6 (t'), 78.0 (d'), 116.0 (d'), 122.1 (d'), 122.2 (s'), 127.2 (d'), 130.0 (d'), 143.4 (s'), 146.9 (d'), 153.9 (s'), 162.2 (s'), 168.1 (s'), 194.5 (s'); exact mass *m/z* calcd for $C_{23}H_{31}NO_6SSi$ 477.1641, found 477.1640.

Methyl 2-[3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]-2-methoxypyrid-4-yl]ethanoate (2.3).



Bu_3SnH (1.31 mL, 4.88 mmol) and AIBN (50 mg) were added to a solution of **9.9** (1.55 g, 3.25 mmol) in PhMe (50 mL). The mixture was refluxed for 2 h, and then the solvent was evaporated. Flash chromatography of the residue over silica gel (3 x 15 cm), using 1:4 EtOAc-hexanes, gave **2.3** (1.05 g, 99%): FTIR (CH_2Cl_2 cast) 837, 1063, 1081, 1576, 1598, 1744 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.06 (s, 6 H), 0.90 (s, 9 H), 3.70 (s, 3 H), 3.84 (s, 2 H), 3.95 (s, 3 H), 4.79 (s, 2 H), 6.80 (d, J = 6.0 Hz, 1 H), 8.04 (d, J = 6.0 Hz, 1 H); ^{13}C NMR: δ -5.4 (q'), 18.3 (s'), 25.9 (q'), 37.4 (t'), 52.1 (q'), 53.5 (q'), 56.5 (t'), 119.3 (d'), 121.7 (s'), 145.0 (d'), 145.7 (d'), 161.9 (s'), 171.0 (s'); exact mass *m/z* calcd for $C_{15}H_{24}NO_4Si$ (M - CH_3) 310.1475, found 310.1460. Anal. Calcd. for $C_{16}H_{27}NO_4Si$: C, 59.04; H, 8.36; N, 4.30. Found: C, 58.95; H, 8.38; N, 4.24.

5 REFERENCES AND FOOTNOTES

1) The Beilstein database was searched for substructure **1.2**, in which C, C, and O were allowed to have any type of substituents. Related *bicyclic* structures, relevant to the synthesis of camptothecin, are known: *Cf.* Comins, D. L.; Saha, J. K. *Tetrahedron Lett.* **1995**, *36*, 7995.

2) Victory, P.; Borell, J. I.; Vidal-Ferran, A. *Heterocycles* **1993**, *36*, 769.

3) Shariff, A.; McLean, S. *Can. J. Chem.* **1983**, *61*, 2813.

4) Seide, O. *Ber.* **1924**, *57*, 791.

5) (a) Lappin, G. R.; Slezak, F. B. *J. Am. Chem.*, *Soc.* **1950**, *72*, 2806. (b) Roe, A.; Seligman, R. B. *J. Org. Chem.* **1955**, *20*, 1729.

6) Norman, M. H.; Minick, D. J.; Martin, G. *J. Heterocyclic Chem.* **1993**, *30*, 771. *Cf.* Hargrave, K. D.; Proudfoot, J. R.; Grozinger, K. G.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Maudlin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Pal, K.; Skiles, J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Campbell, S. J.; Rosenthal, A. S.; Adams, J. *J. Med. Chem.* **1991**, *34*, 2231.

7) (a) Morisawa, Y.; Kataoka, M.; Sakamoto, T.; Nagahori, H.; Kitano, N. *J. Med. Chem.* **1978**, *21*, 194. (b) Weglinski, Z.; Talik, T. *Rocz. Chem.* **1977**, *51*, 2401.

8) *Cf.* Baumgarten, H. E.; Chien-fan Su, H. *J. Am. Chem. Soc.* **1952**, *74*, 3829.

9) (a) Bobbit, J. M.; Scola, D. A. *J. Org. Chem.* **1960**, *25*, 560. (b) Katritzky, A. R.; Rachwal, S.; Smith, T. P.; Steel, P. J. *J. Heterocyclic Chem.* **1995**, *32*, 979.

10) *Cf.* **8.3** had: FTIR (CH_2CL_2 cast) 1074, 1607, 1732 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.66 (s, 2 H), 3.96 (s, 3 H), 5.37 (s, 2 H), 6.74 (d, J = 4.0 Hz, 1 H), 8.08 (d, J = 4.0 Hz, 1 H); ^{13}C NMR: δ 34.5 (t'), 53.7 (q'), 65.4 (t'), 113.7 (s'), 115.5 (d'), 141.9 (s'), 147.0 (d'), 159.3 (s'), 168.8 (s'); exact mass *m/z* calcd for $C_9H_9NO_3$ 179.0582, found 179.0583. Anal. Calcd. for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.06; H, 4.94; N, 7.74.

11) Dailey, O. D.; Fuchs, P. L. *J. Org. Chem.* **1980**, *45*, 216.

12) Compound **10.2** had: FTIR (CH_2Cl_2 cast) 1732, 1739, 2228, 3428 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.50 (s, 3 H), 2.4-2.9 (br, 1 H), 5.45 (d, J = 6.8 Hz, 1 H), 6.74 (d, J = 6.8 Hz, 1 H); exact mass *m/z* calcd for C_7H_6NO 134.0480, found 134.0478. Anal. Calcd. for C_7H_6NO: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.32; H, 4.31; N, 20.55.